SMB

XXXII CONGRESO NACIONAL DE BIOQUÍMICA

4 - 9 de noviembre, 2018 - Ixtapa, Zihuatanejo, Gro.

PROGRAMA

COMITÉ ORGANIZADOR

Irene Castaño Navarro • David Romero Camarena • Jorge Luis Folch • Soledad Funes

Comité Organizador y
Mesa Directiva 2017 - 2019

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XXXII Congreso Nacional de Bioquímica

4 al 9 de noviembre, 2018 Zihuatanejo, Guerrero

Mensaje del Presidente

Estimados Colegas:

¡Bienvenidos a Ixtapa-Zihuatanejo! A nombre de la Mesa Directiva de la Sociedad Mexicana de Bioquímica y del resto del comité organizador, les damos la más cordial bienvenida a esta hermosa ciudad en la costa del estado de Guerrero. En esta ocasión, la mesa directiva de la SMB eligió este bello lugar para celebrar nuestra gran reunión académica bienal, en la que nos actualizaremos, aprenderemos, compartiremos y discutiremos nuestros trabajos y avances en el conocimiento de las diferentes ramas de la bioquímica.

El nombre de Ixtapa-Zihuatanejo se deriva de la palabra náhuatl Cihuatlan (lugar donde abundan las mujeres) e Ixtapa que significa lugar blanco. Zihuatanejo constituyó desde épocas pre-coloniales un lugar de recreo para los monarcas tarascos. A partir de la conquista, Hernán Cortés envió carpinteros a Zihuatanejo para que se construyeran 3 barcos con las finas maderas del lugar (cedro rojo y roble), y en octubre de 1527 los 3 barcos zarparon rumbo a las Filipinas, lo que inauguró a Zihuatanejo como puerto mercante. Posteriormente, Zihuatanejo se afianzó como exportador de maderas finas y la playa donde se cargaban los barcos que las transportaban aún se llama "La Madera".

En los años 70s Zihuatanejo empezó a aumentar su capacidad hotelera; sin embargo, aún conserva su apariencia e identidad de villa de pescadores que adquirió entre 1890 y 1910. A mediados de los años 70 el gobierno federal comenzó el desarrollo hotelero de Ixtapa, ubicado a solo 7 km de distancia de Zihuatanejo, el cual ahora es un importante destino turístico moderno, con zonas comerciales y una marina. Así, Ixtapa y Zihuatanejo son dos pequeñas ciudades muy distintas entre sí que ofrecen al visitante una gama de atractivos y hermosas playas.

Para la celebración del XXXII Congreso Nacional de Bioquímica hemos preparado un amplio y estimulante programa académico que nos permitirá continuar con la extraordinaria labor que la SMB ha llevado a cabo por más de 60 años, que es impulsar, favorecer y estimular el desarrollo de las diferentes ramas de la bioquímica en México para beneficio de los estudiantes, profesores e investigadores que incursionan en esta área del conocimiento.

En esta ocasión, iniciaremos el Congreso con dos extraordinarias conferencias. La primera será dictada por el Dr. Antonio Lazcano Araujo, miembro de El Colegio Nacional, en la cual hablará sobre Frankenstein y Mary Wollstonecraft Shelley y cómo la novela refleja el proceso de secularización de la biología. La segunda conferencia de la noche estará a cargo de la Dra. Jo Handelsman directora del Wisconsin Institute for Discovery de la Universidad de Wisconsin en Madison-EUA, y asesora científica del presidente Obama, quién nos hablará sobre la comunicación química entre las células que forman comunidades microbianas.

Además, la mesa directiva ha preparado un estimulante programa académico en el que aparte de las conferencias inaugurales, habrá 10 conferencias plenarias, incluyendo la de clausura, todas ellas dictadas por líderes académicos tanto de México como del extranjero. Además se llevarán a cabo 9 simposia que versarán sobre las ramas de la bioquímica que componen a la SMB y un simposio bilateral Hispano-Mexicano sobre Epigenética y Biología de Sistemas. También habrá 21 sesiones de presentaciones orales en las que participarán 123 estudiantes, 3 sesiones de "charlas de elevador" en las que presentarán 45 estudiantes un resumen muy breve de sus trabajos con el objeto de darle difusión a sus carteles. Tendremos además la presentación de 800 carteles (aproximadamente 200 por día). Adicionalmente hemos organizado 11 sesiones pequeñas que hemos llamado "Having coffee with…" en las cuáles todos los invitados a conferencias plenarias así como los extranjeros del simposio bilateral se reunirán con aproximadamente 10 estudiantes donde tendrán la oportunidad de acercarse a ellos para hablar de sus temas de investigación. También habrá un curso breve de redacción de artículos científicos impartido por el Dr. Gabriel Gasque, *Senior Editor* en *PLOS Biology*.

Quisiera enfatizar que la mayoría de los participantes (67%) son estudiantes tanto de posgrado como de licenciatura que vienen de 29 estados del país, lo cual hemos fomentado otorgando un gran número de becas.

Estamos convencidos de que este Congreso será un foro en el que los jóvenes podrán comunicar sus resultados y se enriquecerán con los comentarios y sugerencias de los investigadores más establecidos, y además surgirán nuevas oportunidades de colaboración.

Estoy segura de que todos los participantes disfrutaremos enormemente este Congreso, dada la alta calidad académica, así como la variedad, calidad y rigurosidad de sus temas y ponentes. Además de que servirá para estrechar los lazos de colaboración y amistad entre los miembros de nuestra comunidad.

Irene Beatriz Castaño Navarro
Presidente SMB 2017-2019

El Comité Organizador desea expresar especialmente
su reconocimiento y agradecimiento a:

Al Instituto de Fisiología Celular, UNAM
Por su invaluable y continuo apoyo a la Sociedad Mexicana de Bioquímica

A la Lic. María Teresa Castillo Martínez
por su generoso, constante y atinado apoyo a la Sociedad Mexicana de Bioquímica

A la Lic. Diana Verónica Cordero Tavares
Por su atinada intervención durante el proceso de la Comisión de Admisión, así como en la coordinación de las finanzas.

A la Bióloga Andrea Ortiz Arcos
Por su dedicación e incondicional apoyo en las diferentes etapas de organización del congreso así como su esmero con el control y verificación del sistema de facturación, así como la creatividad en los diseños que se le encomendaron.

Al Ing. Juan Manuel Barbosa Castillo
Por la elaboración de la memoria electrónica y soporte técnico brindado.

A Ricardo y Omar Chávez Castillo
Por su valioso apoyo y trabajo realizado en las diferentes etapas de la coordinación y organización de este congreso.

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POSTER SESSIONS

XXXII NATIONAL CONGRESS OF BIOCHEMISTRY
Ixtapa-Zihuatanejo, Gro
November 4-9, 2018

Hrs	Sunday 4	Monday 5	Tuesday 6	Wednesday 7	Thursday 8	Friday 9
8:30-9:30		**Brendan Cormack Plenary lecture 2**	**Jean Philippe Vielle Plenary lecture 4**	**Susana López Charreton Plenary lecture 6**	**Heather Pinkett Plenary lecture 8**	
9:30-11:00		Oral Presentations 1, 2, 3	Oral Presentations 7, 8, 9	Oral Presentations 13, 14, 15	Oral Presentations 19, 20, 21	
11:00-11:30	Registration 11:00-18:00	**COFFEE BREAK**				Out
11:30-13:00		Oral Presentations 4, 5, 6 & simultaneous Technical Conferences AUREUS TERMOFISHER	Oral Presentations 10, 11, 12 & simultaneous Technical Conferences GE Healthcare Life Scie. BIO-RAD Life Science	Oral Presentations 16, 17, 18 & simultaneous Technical Conferences UNIPARTS AGILENT Tech	**Lars Steinmetz Plenary Lecture 9 11:30-12:30**	
13:00-13:15			**Group photograph 13:00-13:15**		**Group photograph 12:30-12:45**	
13:00-14:30		**LUNCH**				
14:30-16:30		**Simultaneous Symposia** 1. Molecular Biology of Fungi 2. Biochemistry and Molecular Biology of Bacteria 3. Reactive Oxygen Species & Signal Transduction	**Simultaneous Symposia** 4. Bioenergetic & Protein Structure and Function 5. Biochemistry and Plant Molecular Biology 6. Molecular Biology of Viruses	**Simultaneous Symposia** 7. Hispano-Mexicano: Epigenetic and Systems Biology 8. Neuroscience & Neurobiology 9. Cultural	Poster Session 4 **14:30-16:30**	
16:30-17:30		**Johannes Herrmann Plenary lecture 3**	**Andrew Dillin Plenary lecture 5**	**Arturo Alvarez Buylla Plenary lecture 7**	**Malcolm Whitman Closing Lecture**	
17:30-18:00		Elevator talks	Elevator talks	Elevator talks	**Final announcements & closing ceremony**	
18:00-20:00	**Antonio Lazcano Opening Lecture 18:00-19:00 Jo Handelsman Plenary lecture 19:00-20:00**	**Poster Session 1 18:00-20:00** Having coffee with…**18:00-19:00** (simultaneous with Posters)	**Poster Session 2 18:00-20:00** Having coffee with…**18:00-19:00** (simultaneous with Posters)	**Poster Session 3 18:00-20:00** Having coffee with…**18:00-19:00** (simultaneous with Posters)	**Closing dinner 21:00-24:30**	
20:00-23:00	**Welcome cocktail 20:00-23:00**		**Business session 20:00 - 21:00**			

PROGRAM of EVENTS

Sunday November 4, 2018

11:00 – 18:00 Registration
Centro de Convenciones Azul Ixtapa

Opening Ceremony, Plenary Lectures

Zeus Room

17:30 – 18:00 Opening Ceremony

18:00 – 19:00 **Opening Lecture**
El Golem, Frankenstein y la secularización de la biología
Antonio Lazcano Araujo
Miembro de El Colegio Nacional
Universidad Nacional Autónoma de México

Chair: *David Romero Camarena*
Centro de Ciencias Genómicas, UNAM

19:00 – 20:00 **Plenary Lecture**
Secondary Metabolites in Microbial Communities
Jo Handelsman
University of Wisconsin. Madison USA

Chair: *Susana Brom*
Centro de Ciencias Genómicas, UNAM

19:30 – 21:30 Welcome Cocktail
Garden Olimpo CC Azul Ixtapa

Oral presentations will be held in the Convention Center Azul Ixtapa.

Poster presentations will be held in the Ixtapa Room, Hotel Azul Ixtapa

MONDAY

Monday November 5, 2018

Zeus Room

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8:30 – 9:30 **Plenary Lecture**

NAD$^+$ and the regulation of virulence in the yeast pathogen *Candida glabrata*

Basil Hussain, Shih-Jung Pan, Zhuwei Xu, Elizabeth Hwang-Wong, Brian Green, Nicole Benoit, Carlos Gomez and **Brendan Cormack**

Johns Hopkins University. School of Medicine. Department of Molecular Biology and Genetics. USA

Chair: *Alejandro de las Peñas*
Instituto Potosino de Investigación Científica y Tecnológica

Simultaneous Sessions

9:30 – 11:00	**1. Reactive Oxygen Species** **Zeus Room I**	**2. Genetics, Epigenetics & Genetic Regulation I** **Zeus Room II**	**3. Signal Transduction and Cell Differentiation I** **Zeus Room III**
	Chair: *Vianey Olmedo* U. GTO.	Chair: *Alfredo Herrera* LANGEBIO CINVESTAV	Chair: *Vanesa Olivares* UASLP
9:30-9:45	**Dioxygen Avoidance Theory: An experimentally validated mathematical model for ROS-mediated cell differentiation.** *Wilhelm Hansberg.* Instituto de Fisiología Celular, UNAM	**Zinc dependent and HIZR-1 mediated activation of HLH-30 and the autophagy-lysosomal pathway is required for zinc homeostasis.** *Ciro Cubillas*. Washington University	**Identification of Novel Substrates of Protein Tyrosin Phosphatase 1B in Breast Cancer Cells Through SILAC-based Phosphoproteomics.** *Luis Enrique Arias Romero.* FES Iztacala, UNAM
9:45-10:00	**Influence of redox signaling on the regulation of the Erk1/2 kinase and activation of the transcription factor Nrf2 in hearts with post-conditioning.** *Arturo Macías-López*. Instituto Nacional de Cardiología "Ignacio Chávez "	**Quantitation of the relative protein levels of *Debaryomyces hansenii* catalases A and T through their fusion to a fluorescent reporter.** *Brandon Bustos-García*. Facultad de Ciencias, UNAM	**Role of pALT$^{Ink4a/b}$ in mouse embryonic fibroblasts during the establishment of cellular senescence.** *Miguel del Ángel Muñoz.* Cellular Physiology Institute, UNAM
10:00-10:15	**Transcriptome analysis of the antagonistic effect of NADPH oxidase gene RbohB in the common bean after rhizobial and arbuscular mycorrhizal inoculation.** *Citlali Fonseca-García*. Instituto de Biotecnología, UNAM	**Analysis of the *Physcomitrella patens* methylation machinery mutant on development and stress response.** *Laura Esther Estrada Martinez.* Universidad Autónoma de San Luis Potosí	**Transcription analysis of endothelial cells treated with tumor soluble factors of cell line ZR-7530.** Andrei Ivan Favela Orozco. Instituto de Investigaciones Biomédicas, UNAM
10:15-10:30	**A LORELEI characterization during the Rhizobium-legume interaction.** *Pascual Morales Edgar J.* Instituto de Biotecnología. UNAM	**Inaccurate microRNA detection in every cell culture due to Fetal Bovine Serum.** *Carlos Fabián Flores-Jasso.* Instituto Nacional de Medicina Genómica	**The importance of cell context: Ski and SnoN regulatory mechanisms in hepatocytes.** *Diana G. Ríos-López*. Instituto de Fisiología Celular. UNAM
10:30-10:45	**Global gene expression analysis reveals a persistent activation of NRF2 in liver carcinogenesis: Implications in cancer drugs resistance.** *Julio Isael Pérez-Carreón*. INMEGEN	**Optogenetics applied to PLK4, the master regulator of the centrosome.** *Eduardo Brito-Alarcón.* Instituto de Biotecnología, UNAM	**Wnt pathway-related Extracellular Antagonists expression in osteoblast-like cells.** Alma Y. Parra-Torres. Instituto Nacional de Medicina Genómica
10:45-11:00	**Development of monitoring oxygen and carbon dioxide transfer rate (OTR and CTR) device and cotton closure simulation during *Pichia pastoris* shake flask cultures.** *Daniel Cabrera Santos.* Instituto de Investigaciones Biomédicas, UNAM	**Myelin and Lymphocyte Protein (MAL) induces MUC1 lysosomal degradation.** *Antonio Roberto Lara Lemus*. Instituto Nacional de Enfermedades Respiratorias "Ismael Cosío Villegas".	

Simultaneous Sessions

11:30 – 13:00	**4. Biotechnology I** **Zeus Room I**	**5. Systems Biology & Bioinformatics I** **Zeus Room II**	**6. Medicine, Health & Nutrition I** **Zeus Room III**
	Chair: *Luis Gerardo Treviño Quintanilla.* UPEMOR	Chair: *Gabriel del Rio* IFC UNAM	Chair:*Claudia Segal Kischinevzky*. FC UNAM
11:30-11:45	***Pseudomonas chlororaphis* as a new model for shikimic acid production.** *Alma Yolanda Alva Aviles*. Instituto de Biotecnología, UNAM	**Identification and Classification of Single Nucleotide Polymorphisms as Biomarkers associated with Dementia through Data Mining and Machine Learning.** *Erick Cuevas Fernández*. Facultad de Medicina, UAEM	**Identification and selection of genetic biomarkers for atherosclerosis.** *José Javier Álvarez Arroyo*. UAEM
11:45-12:00	**Bacterial biodegradation of endocrine disruptors di-(2-ethylhexyl) phthalate and di-isononyl phthalate.** *Marco Antonio Pereyra Camacho*. Instituto Potosino de Investigación Científica y Tecnológica A.C.	**Evidence of the Red-Queen Hypothesis from Accelerated Rates of Evolution of Genes Involved in Biotic Interactions in *Pneumocystis*.** *Luis Delaye*. CINVESTAV Irapuato	**Establishment of *ex vivo* and *in vivo* models for the study of bone remodeling.** *Brenda Ivette Iduarte-Frias*. CICESE Ensenada
12:00-12:15	***Aspergillus sydowii*, a halophile fungus with potentials to degrade lignocellulose, polycyclic aromatic hydrocarbons and pharmaceuticals: a transcriptomic and biochemical view.** *Ramón Alberto Batista-García*. UAEM	**Modeling the structure-function relationship in biological systems with machine learning.** *Fernando Fontove*. Instituto de Fisiología Celular, UNAM	**Profibrotic Role of Matrix Metalloproteinase-28 and Potential Diagnostic Biomarker for Idiopathic Pulmonary Fibrosis.** *Mariel Maldonado*. Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas
12:15-12:30	**Rational design of chimeric endolysins with application in aquaculture.** *César Salvador Cardona--Félix*. IPN	**Modeling the regulatory network of lymphopoiesis.** *Luis Mendoza Sierra*. Instituto de Investigaciones Biomédicas, UNAM	**Aging and absence of Zmpste24 protects premature aging mice from developing bleomycin-induced pulmonary fibrosis through the overexpression of antifibrotic microRNAs.** *Jazmín Calyeca*. Facultad de Ciencias. UNAM
12:30-12:45	**An orthogonal system for beta-branching in polyketide biosynthesis.** *Pablo Cruz-Morales*. Joint Bioenergy Institute. USA	**The cellular mechanisms of lifespan extension by metformin.** *Michelle Crisely Munguía Figueroa*. LANGEBIO. CINVESTAV Irapuato	**Restoration of circadian levels of NAD+ reduces physiopathology caused by a high-fat diet.** *Quetzalcoatl Escalante Covarrubias*. IIB-UNAM
12:45-13:00		**Effect of antibiotics in the ecological dynamic of microbial communities.** *Daniela Reyes González*. Centro de Ciencias Genómicas, UNAM	**Omega-3 fatty acids in diabetes.** *Ricardo Mejía Zepeda*. FES Iztacala, UNAM

11:00 – 11:30 Coffee break Foyer CC Azul Ixtapa

11:30 – 13:00 Technical Conferences
Zeus Room IV

Chair: *Carlos Wong Baeza*
Escuela de Ciencias Biológicas, IPN

11:30 – 12:15 AUREUS
Aplicaciones de microfluidica para el análisis de células individuales
Luis Roberto Zaldivar Rivera

12:15 – 13:00 TERMOFISHER
Herramientas para la determinación de Proteína A y Agentes adventicios en productos Biotecnológicos
Mariana Pérez Escobar

13:00 – 14:30 Lunch

Simultaneous Symposia 1
14:30 – 16:30

Zeus Room I

Molecular Biology of Fungi

Chair: *Alejandro de las Peñas*
Instituto Potosino de Investigación Científica y Tecnológica

14:30 – 15:00 Mycobiota of Greenland Ice Sheet for a more sustainable world
Nina Gunde-Cimerman
Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia

15:00 – 15:30 The components of the fungal cell wall change the polarization state of human macrophages
Emmanuel Orta Zavalza, Brenda Lizbeth Estrada Capetillo and Angel Luis Corbi Lopez
Departamento de Ciencias Quimico-Biologicas, Universidad Autonoma de Ciudad Juarez. Ciudad Juárez, Chihuahua

15:30 – 16:00 MYO-5 the single actin-associated molecular motor in *Neurospora crassa*
Arianne Ramírez-del Villar, Robert W. Roberson, Olga A. Callejas-Negrete, **Rosa R. Mouriño-Pérez**
Departamento de Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada. Ensenada, Baja California

16:00 – 16:30 IRES-dependent translated genes in fungi: Computational prediction, phylogenetic conservation and functional association
Enrique Merino, Liliana Pardo and Esteban Peguero-Sanchez
Instituto de Biotecnología, UNAM

Simultaneous Symposia 2
14:30 – 16:30

Zeus Room II

Biochemistry and Molecular Biology of Bacteria

Chair: *Lourdes Girard*
Centro de Ciencias Genómicas, UNAM

14:30 – 15:00 Conjugative transfer of rhizobial plasmids in plant nodules
Susana Brom, Luis Alfredo Bañuelos-Vázquez, Gonzalo Torres Tejerizo, Lourdes Girard, Laura Cervantes de la Luz and David Romero
Centro de Ciencias Genómicas, UNAM

15:00 – 15:30 Identification of small RNAs and small peptides in *E. coli.*
Juan Miranda Ríos and Gisela Storz
Unidad de Genética de la Nutrición. Instituto de Investigaciones Biomédicas UNAM and Instituto Nacional de Pediatría

15:30 – 16:00 Life in a microbial community: get by with a little help from your friends
Gabriela Olmedo Álvarez
Centro de Investigación y de Estudios Avanzados. Instituto Politécnico Nacional. Unidad Irapuato

16:00 – 16:30 Distinctive microbiome in the southwestern Gulf of Mexico, a historically impacted zone
Tina Godoy, Alejandra Escobar, Luciana Raggi, Adolfo Gracia, Rosa María Gutierrez, Katy Juárez, Enrique Merino, Lorenzo Segovia, Alexei Licea, Alejandro Sánchez, **Liliana Pardo-López**
Instituto de Biotecnología, UNAM

Simultaneous Symposia 3
14:30 – 16:30

Zeus Room III

Reactive Oxygen Species & Signal Transduction

Chair: *María Viridiana Olin Sandoval*
Instituto Nacional de Ciencias Médicas y Nutrición

14:30 – 14:55 Metabolic modeling and control analysis of *Trypanosoma cruzi* antioxidant defense
Emma Saavedra, Citlali Vázquez, Zabdi González-Chávez
Department of Biochemistry. Instituto Nacional de Cardiología

14:55 – 15:20 Modulating the redox state: an alternative to modify the behavior of senescent cells in the aging brain
Mina Königsberg
Departamento de Ciencias de la Salud, División de Ciencias Biológicas y de la Salud. Universidad Autónoma Metropolitana-Iztapalapa

15:20 – 15:45 Molecular Regulation of Renal Salt and Potassium Metabolism
Gerardo Gamba
Instituto Nacional de Ciencias Médicas y Nutrición
Instituto de Investigaciones Biomédicas, UNAM

15:45 – 16:10 Molecular Mechanisms of Insulin Resistance induced by Fatty Acids
Jesus Alberto Olivares-Reyes, Adriana Roura-Guiberna, J. Gustavo Vazquez-Jimenez and Judith-Hernandez Aranda
Department of Biochemistry, CINVESTAV-IPN

16:10 – 16:30 An alternative polyamine pathway mediates lysine plasticity in response to H2O2 stress in *Saccharomyces cerevisiae*
Viridiana Olin Sandoval, Leonor Miller-Fleming, Robert Haas, Markus Ralser.
Department of Food Science and Technology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán
Department of Biochemistry, University of Cambridge

Zeus Room

16:30 – 17:30 **Plenary Lecture**

Mitochondrial protein biogenesis: A huge challenge for eukaryotic cells

Johannes M. Herrmann

Cell Biology, University of Kaiserslautern. Kaiserslautern, Germany

Chair: *Soledad Funes*
Instituto de Fisiología Celular, UNAM

17:30 – 18:00 **Elevator talks**
Chosen from poster presentations

SB-10 Identification of trans-acting riboswitches in bacterial genomes. **Edgar Rodríguez García**

SB-17 Identification and analysis of the genes involved in the biosynthetic pathways of cellulose and lignin in *Agave tequilana* Weber. **Luis Fernando Maceda López**

B-29 The conformational landscape of proteins: study of de novo designed TIM barrels. **Sergio Romero-Romero**

BT-11 Overexpression of dehydrin DHN1 and rubisco activase in maize plants transformed by the intragenic method. **Raymundo Belmont Valadez**

BT-35 Prospecting and characterization of psychrophiles microorganisms plant growth promoters. **Irán Tapia Vázquez**

GR-11 DNA methylation alterations in adipocytes derived from obese diabetic patients. **Federico Centeno Cruz**

GR-20 Characterization of epigenetic changes associated with the metabolic memory *In vitro*. **Martí David Wilson Verdugo**

GR-29 Stress induced expression of *SLM35* in *Saccharomyces cerevisiae*. **Hernán Romo-Casanueva**.

IP-16 Recombinant protein harboring relevant epitopes from Influenza A H1N1, as a new candidate vaccine against flu. **Karen Lizbeth Reyes-Barrera**

MH-16 Biological implications of the proteins and microRNAs contained in hepatocellular carcinoma cells-derived extracellular vesicles. **Luis Alberto Castro Sánchez**

MV-6 Functional analysis of Rep and CP promoters of a natural mutant of Tomato yellow leaf curl virus (TYLCV). **Jesús Aarón Avalos-Calleros**

MV-24 Molecular characterization, phylogenetic analysis and replication of a new begomovirus infecting bean in Nayarit, Mexico. **Martínez-Marrero Nadia**

ST-10 Identification of the Guanine Nucleotide Exchange Factor VAV1 as a Novel Target of the Protein Tyrosine Phosphatase 1B. **Olga Villamar Cruz**

ST-22 The G-protein β subunit, Gpb1, regulates virulence in *Mucor circinelloides*. **Marco Iván Valle-Maldonado**.

18:00 – 19:00 **Having coffee with…..**
Round Tables for students. Chosen from oral presentations
Artemisa, Venus & Executive Rooms. First Floor CC Azul Ixtapa

18:00 – 20:00 Poster Session I
Ixtapa Room, Hotel Azul Ixtapa

SB	SYSTEMS BIOLOGY & BIOINFORMATICS I
B	BASIC BIOCHEMISTRY I
BT	BIOTECHNOLOGY I
GR	GENETICS, EPIGENETICS AND GENETIC REGULATION I
IP	IMMUNOLOGY & PARASITOLOGY I
MH	MEDICINE HEALTH & NUTRITION I
MV	MICROBIOLOGY & VIROLOGY I
ST	SIGNAL TRANSDUCTION I

TUESDAY

Tuesday November 6, 2018

Zeus Room

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8:30 – 9:30 **Plenary Lecture**

From sex to apomixis: evolution, control, and induction of clonal reproduction

Jean Philippe Vielle

Grupo de Desarrollo Reproductivo y Apomixis
UGA LANGEBIO CINVESTAV Irapuato

Chair: *Stewart Gillmor*
LANGEBIO CINVESTAV Irapuato

Simultaneous Sessions

9:30 – 11:00	**7. Genetics, Epigenetics & Genetic Regulation II** **Zeus Room I**	**8. Microbiology** **Zeus Room II**	**9. Toxicology & Pharmacology** **Zeus Room III**
	Chair: *Carlos Fabián Flores Jasso* INMEGEN	Chair: *Susana Brom* CCG UNAM	Chair: Verónica Morales Tlalpan Universidad Autónoma de Querétaro
9:30-9:45	**Defensin γ-Thionin from Capsicum chinense induces apoptosis in the human breast cancer cell line MCF-7 and regulate histone H3 epigenetic modifications.** *María Teresa Arceo-Martínez*. Universidad Michoacana	**Mixing synthetic ecology and metagenomics towards mine waste bioremediation.** *Luis D. Alcaraz*. Facultad de Ciencias, UNAM	**Phytochemical scrutiny and evaluation of the biological activity of wood extracts.** *Malinali Ambriz-Ortiz*. Universidad Michoacana de San Nicolás de Hidalgo
9:45-10:00	**CTCF-KDM4A complex correlates with histone modifications that negatively regulate the tumor suppressor CHD5 gene expression in cancer cell lines.** *Lissania Guerra-Calderas*. Universidad Autónoma Metropolitana	**Strategy to identification of mutations in resistant *Mycobacterium tuberculosis* isolates in Jalisco Mexico.** *Ikuri Álvarez-Maya.* Center for Research and Assistance in Technology and Design of the State of Jalisco. CIATEJ	**Characterization of the interaction of the antimicrobial peptides Pin2 and Pin2 [GVG] using lipid membrane model systems.** *Brandt Bertrand*. Instituto de Ciencias Físicas, UNAM
10:00-10:15	**CTCF regulates IL6 expression in breast cancer cells.** *Gustavo Ulises Martinez-Ruiz*. Hospital Infantil de México Federico Gómez. UNAM	**Characterization of a putative TCS implicated in the response to salinity stress in *Rhizobium etli* CFN42.** *David Correa-Galeote*. Centro de Ciencias Genómicas, UNAM	**FIG1-dependent cell death during cell cycle arrest in yeast.** *Vladimir Juarez A*. Instituto de Fisiología Celular, UNAM
10:15-10:30	**Methylation pattern of the human ATP2A3 gene promoter in gastric and colon cancer cell lines.** *Angel Zarain-Herzberg.* School of Medicine, UNAM	**Identification and characterization of a family of outer membrane proteins of *Helicobacter pylori*, which scavenges iron from human sources.** *José de Jesús Olivares-Trejo.* Posgrado en Ciencias Genómicas. UACM	***Moringa oleifera* seed prolamin fraction and its inhibitory capacity on the angiotensin converting enzyme-I.** *Jesús Ricardo Pérez Velázquez*. Facultad de Ciencias Químicas de la Universidad Juárez del Estado de Durango
10:30-10:45	***Candida glabrata cis*-acting element Sil2126 negatively regulates the expression of adhesin-encoding genes through chromatin loop formation.** *Eunice López-Fuentes*. IPICyT	**Inhibition of *las* quorum sensing system is not enough to suppress virulence of *Pseudomonas aeruginosa.*** *Martín Paolo Soto-Aceves*. Instituto de Investigaciones Biomédicas, UNAM	**Lupresan, a new drug that prevents or reverts the formation of lipidic particles that trigger a lupus mouse model.** *Carlos Wong Baeza.* Escuela Nacional de Ciencias Biológicas, IPN
10:45-11:00	**Genetic analysis of aging factor SWR1 in *Saccharomyces* and *Drosophila*.** *Judith Ulloa Calzonzin*. LANGEBIO CINVESTAV Irapuato	**Strategies of *Avibacterium paragallinarum* to successful colonize host tissues.** *Candelario Vázquez Cruz*. Centro de Investigaciones en Ciencias Microbiológicas. BUAP	**Highly purified extracellular vesicles from Fetal Bovine Serum reveal a mutually exclusive two-set of microRNAs.** *Filiberto A. Bautista-Moreno*. INMEGEN

Simultaneous Sessions

11:30 – 13:00	**10. Basic Biochemistry I** **Zeus Room I**	**11. Biotechnology II** **Zeus Room II**	**12. Medicine, Health & Nutrition II** **Zeus Room III**
	Chair: *Leonardo Peraza* IFC UNAM	Chair: *Gloria Yepiz, CIAD*	Chair: *Ernesto Soto Reyes* UAM
11:30-11:45	**Multi-protein regulation of intracellular pH during human sperm capacitation.** *Arturo Matamoros-Volante*. Instituto de Biotecnología, UNAM	**Folates and N2 fixation: Compartmentalized distribution of folates derivatives in mitochondria, plastids, and *Rhizobium* from *Phaseolus vulgaris* nodules in relation with N2 fixation levels.** *Wendy Judith Berdeja Zamudio*. Tecnólogico de Monterrey	**Expression of PD1 and TIM3 in the inflammatory peritumoral infiltrate of patients with breast cancer.** *Miguel Angel Fonseca-Sánchez*. Departamento de Genética, Hospital General de México Dr. Eduardo Liceaga
11:45-12:00	**Effect of Ergosterol in the Tyrosine residue of the Bacilomycine D as a basis for studies of their interaction with lipid membranes.** *José Francisco Espinosa-Romero*. Instituto de Ciencias Físicas, UNAM	**Folate and ethylene metabolic crossroads: Ethylene modulation of folate pools and methionine synthase expression and activity in climacteric fruit.** *Rocío Isabel Díaz de la Garza*. Tecnólogico de Monterrey	**Activation of energy metabolism by estrogen in human breast carcinoma.** *Silvia Cecilia Pacheco Velázquez*. Instituto Nacional de Cardiología "Ignacio Chávez"
12:00-12:15	**The cytochrome b carboxyl-terminal end is a central regulator of the bc_1 complex biogenesis in *Saccharomyces cerevisiae*.** *Daniel Flores-Mireles*. IFC UNAM	**The Intragenic Method for Maize Improvement: Drought Tolerance and Improved Photosynthesis.** *Verónica Garrocho Villegas.* Facultad de Química, UNAM	**LPS-induced cancer progression on triple negative breast cancer cells via nitric oxide signaling pathway mediated by TLR4/NF-κB.** *Karina Ruiseco-Flores*. IF, BUAP
12:15-12:30	**Biochemical basis of PirAvp/PirBvp toxins-receptor interactions: a galactosamine mediated binding in the epithelial cells of hepatopancreas of shrimp.** *Victorio-De Los Santos Marcelo.* CIAD	**Antagonistic activities analysis of *Trichoderma* spp. isolates over *Fusarium graminearum*, fusariosis causal agent on *Triticum aestivum* (wheat).** *Kate Manuela López Hernández*. Instituto Tecnológico Superior de Ciudad Hidalgo	**Antiproliferative effect of molecular iodine and cyclophosphamide in acute lymphoblastic leukemia cells.** *Maritza L. Soria-Ornelas* Instituto de Neurobiología, UNAM
12:30-12:45	**Growth and reserve lipids production with the non-pathogenic *Acinetobacter baylyi* strain ADP1 using different carbon sources.** *Karina J. Salcedo Vite.* IBT UNAM	**Carotenoid/Bacteriochlorophyll ratio during the culture of *Rhodopseudomonas palustris* ATCC1007 at different nitrogen concentration.** *Carlos Jeovanny López Romero*. UPIBI IPN	**Analysis of irisin protein expression, in infiltrating breast cancer, in postmenopausal women with different body mass indexes.** *María Elena Tejeda Hernández*. Facultad de Medicina, UNAM
12:45-13:00	***In vitro* evaluation of the phosphate solubilization efficiency of fungal strains isolated from the rhizosphere of bean, corn and tomato plants.** *Dorcas Zúñiga Silgado.* UAEM	**Bisphenol-A on the growth and enzymatic activity of *Aspergillus fumigatus*.** *Binicio Ramírez-Mendoza*. Faculty of Agrobiology. Autonomous University of Tlaxcala,	**Over-elongation of centrioles in cancer promotes centriole amplification and size deregulation, as recurrent features of cancer cells.** Adan Guerrero. IBT, UNAM

11:00 – 11:30 Coffee break Foyer CC Azul Ixtapa

11:30 – 13:00 Technical Conferences
Zeus Room IV

Chair: *Victor Emmanuel Balderas Hernández*
IPICyT

11:30 – 12:15 GE Healthcare Life Sciences
Cell Mutiparameter Analysis using Fluorescence Microscopy
High-content analysis – Automated imaging and analysis of cellular assays
Sandra Rosa da Silva, Msc
Product Group Manager - Latin America

12:15 – 13:00 Life Science Group, Bio-Rad
Revolucionando el Western blot a través de la multidetección fluorescente
Carlos Bravo

13:00 – 14:30 Lunch

Simultaneous Symposia 4
14:30 – 16:30

Zeus Room I

Bioenergetic & Protein Structure and Function

Chair: *Xóchitl Pérez Martínez*
Instituto de Fisiología Celular, UNAM

14:30 – 15:00 On the conformational stability of natural and *de novo* TIM barrels.
Daniel Alejandro Fernández Velasco, Sergio Romero Romero, Ricardo Muñíz Trejo, Adela Rodríguez Romero, Miguel Costas
Laboratorio de Fisicoquímica e Ingeniería de Proteínas, Departamento de Bioquímica, Facultad de Medicina, UNAM

15:00 – 15:30 Role of mitochondria on the cancer chemoprevention by an adenosine derivative in a model of cirrhosis-hepatocellular carcinoma induced by diethylnitrosamine in rats**.**
Chagoya de Sánchez V, Chávez E, Velasco-Loyden G, Lozano-Rosas MG, Pérez-Carreón JI, Rodríguez-Aguilera JR.
Instituto de Fisiología Celular, UNAM

15:30 – 16:00 Mitochondrial calcium transport and permeability transition in plants.
Manuel Gutiérrez Aguilar
Facultad de Química, UNAM

16:00 – 16:30 Discovery of novel RNase P inhibitors via an activity-binding-structure pipeline
Alfredo Torres-Larios
Department of Biochemistry and Structural Biology, Instituto de Fisiología Celular, UNAM

Simultaneous Symposia 5
14:30 – 16:30

Zeus Room II

Biochemistry and Plant Molecular Biology

Chair: *Maria Luisa Villarreal*
Centro de Investigación en Biotecnología, UAEM

14:30 – 15:00 Common bean microRNAs emerging as regulators of the nitrogen-fixing rhizobia symbiosis
Georgina Hernández, Damien Formey, José A. Martínez-Rodríguez, Bárbara Nova-Franco, Luis P. Íñiguez, Lourdes Girard, José L. Reyes, Luis Cárdenas, Oswaldo Valdés – López, Alfonso Leija, Sara I. Fuentes, Mario Ramírez
Centro de Ciencias Genómicas, UAM

15:00 – 15:30 Understanding plant nutrition for a better human nutrition
David G. Mendoza-Cozatl
Division of Plant Sciences, University of Missouri, Columbia Mo, USA

15:30 – 16:00 Regulation of pattern formation in plant embryogenesis by miRNAs
Stewart Gillmor
LANGEBIO CINVESTAV, Irapuato

16:00 – 16:30 The roles of the *myo*-inositol pathway to ascorbate at conferring plants enhanced growth and abiotic stress tolerance
Argelia Lorence
Arkansas Biosciences Institute, Arkansas State University, USA

Simultaneous Symposia 6
14:30 – 16:30

Zeus Room III

Molecular Biology of Viruses

Chair: *Rosa Ma. Del Ángel*
CINVESTAV IPN Zacatenco

14:30 – 15:00 Adenoviral Replication Compartments Molecular hubs for viral replication and control of virus-cell interactions
Ramón A. González
Centro de Investigación en Dinámica Celular, Universidad Autónoma del Estado de Morelos

15:00 – 15:30 Dynamics of the intestinal virome during the first year of life
Blanca Taboada, Pavel Isa, Susana López, Yarenci Aguado, Angélica Serrano, Patricia Morán, Liliana Rojas, Horacio Pérez, Javier Torres, Cecilia Ximenez, Carlos Arias
Instituto de Biotecnología, UNAM

15:30 – 16:00 Down regulation of antiapoptotic proteins by Feline calicivirus: perspectives to cancer control.
Ana Lorena Gutiérrez Escolano, Oscar Salvador Barrera Vazquez
CINVESTAV IPN

16:00 – 16:30 The nonstructural proteins 3 and 5 from flavivirus modulate nuclearcytoplasmic transport and innate immune response targeting nuclear proteins
Margot Cervantes-Salazar, Ana L. Gutiérrez-Escolano, José M. Reyes-Ruiz, **Rosa M. del Angel**
Departamento de Infectómica y Patogénesis Molecular, CINVESTAV-IPN

Zeus Room

16:30 – 17:30 **Plenary Lecture**

Communication of cellular misfolding across tissues for enhanced longevity

Andrew Dillin

University of California, Berkeley, USA

Chair: *Alexander De Luna*
LANGEBIO CINVESTAV Irapuato

17:30 – 18:00 **Elevator talks**
Chosen from poster presentations

B-30 Deletion of the complex V-dimerizing subunit g and its effect on the mitochondrial bioenergetics in *Ustilago maydis.* **Mercedes Esparza-Perusquía**

B-55 Effect of moderate exercise and metformin treatment on mitochondria isolated from old rats quadriceps. **Stefanie Paola López-Cervantes**

ROS-11 Effect of hypoxia, reoxygenation, temperature and silencing on the expression of white shrimp *Litopenaeus vannamei* manganese superoxide dismutases. **Carlos Ricardo González-Ruiz**

ROS-12 Analysis of the activity of phenolic bean compounds in oxidative stress tolerance in *Caenorhabditis elegans*. **Alejandra Yitzel Guzmán Hernández**.

ROS-14 Stress-induced premature senescence (SIPS) of Primary Prostate Epithelial Cells (HPEC) and Evaluation of Senescence-Associated Secretory Phenotype (SASP). **Verónica Salas-Venegas**

GR-39 AMPK modulates the genetic and metabolic effects of biotin deficiency. **Alain Hernández-Vázquez**

GR-52 Transcriptional divergence in homeologous genes *ALT1* and *ALT2* in *Saccharmomyces cerevisiae*. **Dariel Márquez**

GR-58 Role of ExoA and PolA in *Bacillus subtilis* YwqL-dependent DNA deamination-induced repair.

Jimena Meneses Plascencia

MH-32 Metatranscriptional characterization of the intestinal microbiota in health and obese with metabolic complications children. **Luigui Gallardo-Becerra**

MH-46 Respiratory Tract Microbiota in Patients with Acute Respiratory Infections. **Guerrero Corona Diego**

MV-39 Role of PqsE in the production of pyocyanin and elastase in *Pseudomonas aeruginosa* strain ATCC 9027, which belongs to the outlier PA7 clade. **Selene García-Reyes**

NN-1 Autophagy could cooperate with matrix metalloproteinases to the mouse neural tube closure **Pilar Sarah Acevo-Rodríguez**

NN-19 Prolactin protection against oxidative and hypoxic stress in hippocampal neurons. **Fernando Macías Prado**

T-13 Effects of extract of *Lepidium virginicum* on DNBS-induced colitis in rats. **María Fernanda Durán Vázquez**

T-19 Acute intoxication with sodium nitrate produces hematological and biochemical alterations and expression of hypoxia response genes. **Rebeca Pérez Morales**

18:00 – 19:00 **Having coffee with…..**
Round Tables for students. Chosen from oral presentations
Artemisa, Venus & Executive Rooms. First Floor CC Azul Ixtapa

18:00 – 20:00 Poster Session II
Ixtapa Room, Hotel Azul Ixtapa

B	BASIC BIOCHEMISTRY II
BT	BIOTECHNOLOGY II
ERO	REACTIVE OXYGEN SPECIES I
GR	GENETICS, EPIGENETICS AND GENETIC REGULATION II
MH	MEDICINE, HEALTH & NUTRITION II
MV	MICROBIOLOGY AND VIROLOGY II
NN	NEUROSCIENCES AND NEUROBIOLOGY
T	TOXICOLOGY I

WEDNESDAY

Wednesday November 7, 2018

Zeus Room

8:30 – 9:30 **Plenary Lecture**

Rotavirus strategies to fight-back the antiviral responses of its host-cell

Susana López Charretón

Instituto de Biotecnología, UNAM

Chair: *Ramón González García Conde*
Universidad Autónoma del Estado de Morelos

Simultaneous Sessions

9:30 – 11:00	**13. Neurosciences & Neurobiology** **Zeus Room I**	**14. Genetics, Epigenetics & Genetic Regulation III** **Zeus Room II**	**15. Signal Transduction II** **Zeus Room III**
	Chair: *Carlos Barajas López* IPICYT	Chair: *Eugenio Mancera* CINVESTAV Irapuato	Chair: *Wilhelm Hansberg* IFC, UNAM
9:30-9:45	**Vasoinhibins promote apoptotic cell death in hippocampal neuronal primary cultures.** *Rodrigo Manuel Aroña*. Instituto de Neurobiologia, UNAM	**The TRPV4 cationic channel regulates the transcriptional activity of β-catenin through a direct interaction modulated by its channel activity.** *Refugio García-Villegas*. CINVESTAV IPN	**The role of Voltage-Gated Sodium Channels Subunits in Osteosarcoma Metastasis.** *Nidia Ednita Beltrán Hernández*. Facultad de Medicina. UAEM
9:45-10:00	**Effect of oxidative stress on the P2X7 purinergic receptor and the glycogen synthase kinase transcriptase, in the hippocampus of the rat exposed to low doses of ozone.** *Velázquez-Pérez Raúl*. Facultad de Medicina, UNAM	**Using yeast genetic synthetic lethality to study NPA3 function.** *Martín A. Mora-García*. Instituto de Física, Universidad Autónoma de San Luis Potosí	**Role of Transforming Growth Factor Beta in Neuroblastoma Bone Metastasis**. *Patricia Juárez Camacho.* Centro de Investigación Científica y de Educación Superior de Ensenada
10:00-10:15	**Study of the differences between the proteomic profile of the dorsolateral prefrontal cerebral cortex of individuals who died by suicide and of individuals who died from other causes.** *Manuel Alejandro Rojo Romero*. Fdacultad de Ciencias, UNAM	**The pyrophosphohydrolase RppH is involved in the control of RsmA/CsrA expression in *Azotobacter vinelandii* and *Escherichia coli*.** *Leidy Patricia Bedoya-Pérez*. Instituto de Biotecnología, UNAM	**Tomosyn functions as a PKCδ-regulated fusion clamp in mast cell degranulation.** *Iris K. Madera-Salcedo*. Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán
10:15-10:30	**Prolactin protects rat cortical astrocytes against oxidative stress.** *Miriam Ulloa*. Instituto de Neurobiologia, UNAM	**A long intergenic non-coding RNA is a novel positive regulator of the ABA FIVE-BINDING PROTEIN (AFP1) in *Arabidopsis thaliana*.** *Dhamar Gabriela Rodriguez Tenorio*. Facultad de Ciencias Químicas, UASLP	**Genomic stability is promoted by autophagy and NR4A nuclear receptors.** *Muciño Hernández G*abriel Instituto de Fisiología Celular, UNAM
10:30-10:45	**The role of autophagy and cellular senescence during the development of spinal cord and differentiation of motoneurons.** *Jorge Antolio Domínguez-Bautista*. Institute of Cellular Physiology, UNAM	**Methylation marks on the S-RNase promoter suggest a epigenetic regulation associated with a Gypsy retrotransposon-like sequence.** *Renata Salcedo Sánchez*. Facultad de Química, UNAM	**Characterization of the function of the *non-coding* intermediate *RNA ImncEIN2* in the ethylene perception pathway.** *Jesús Nieto Hernández*. Universidad Autónoma de San Luis Potosí
10:45-11:00	**Cellular systems proposed as a model for the study of dementia.** *Fernanda Anaid Villarreal Reyes.*Facultad de Medicina, UAEM	**Experimental analysis on the *betIBA* operon and *betT1* gene promoters from *Pseudomonas aeruginosa* PAO1.** *Erick Jael Palomo Paz*. Facultad de Química, UNAM	**Natural intra-species variation of the mutational effects of the TOR pathway in cell survivorship**. *J. Abraham Avelar-Rivas.* LANGEBIO CINVESTAV Irapuato

Simultaneous Sessions

11:30 – 13:00	**16. Systems Biology & Bioinformatics II** **Zeus Room I**	**17. Biotechnology III** **Zeus Room II**	**18. Basic Biochemistry II** **Zeus Room III**
	Chair: *Cei Abreu Goodger* LANGEBIO Irapuato	Chair: *Gloria Saab* IBT UNAM	Chair: *Daniel Alejandro Fernández Velasco* FM UNAM
11:30-11:45	**Epikouros: Empowering society with scientific data.** *Gabriel Del Río*. Instituto de Fisiología Celular, UNAM.	**Design of artificial virus-like protein-DNA nanoparticles with programmable self-assembly.** *Carlos Calcines Cruz*. Instituto de Química, UNAM	**Structural studies on the tail domain of the human cytoplasmic Dynein-1.** *Edgar Morales Ríos.* CINVESTAV / Lab. of Molecular Biology MRC. UK
11:45-12:00	**Testing the Domino Theory of Gene Loss in *Buchnera aphidicola*: The Relevance of Epistatic Interactions.** *Luis Delaye*. CINVESTAV Irapuato	**Study of phenolic compounds present in tequila vinasse for its recovery and integral use on biorefinery procesess.** *Cornejo-Corona Ivette*. Centro de Innovación Aplicada en Tecnologías Competitivas	**Structural and thermodynamic contribution of the binding site residues in the lysine-arginine-ornithine binding protein (LAO) to ligand affinity and selectivity.** *Jesús Renán Vergara Gutiérrez*. Facultad de Medicina, UNAM
12:00-12:15	**Experimental evolution of resistance to antibiotics under different intensities of selective pressure.** *Sandra Mayoral Álvarez*. Centro de Ciencias Genómicas, UNAM	**New source of natural bioactive compounds: Phenolic compounds and melanin of residues from energetic plant biomass with a high potential of nutraceutics.** *Domancar Orona Tamayo*. CIATEC, A.C.	**Computational approach to optimize the PTP1B inhibitor JM151.** *Mara Ibeth Campos Almazán***.** Facultad de Medicina y Nutrición. Universidad Juárez del Estado de Durango
12:15-12:30	**IsomiR-index: a method for analyzing quantitatively the isomiR landscape.** *Oscar Omar Moreno-Proo.* Instituto Nacional de Medicina Genómica	**Metabolic engineering to improve hydrogen production by *Escherichia coli*.** *Victor E. Balderas-Hernandez*. IPICyT	**Structural similarity search and biochemical characterization of methicillin resistant *Staphylococcus aureus* shikimate dehydrogenase inhibitors.** *Daniel Isaac Enríquez Mendiola*. Universidad Juárez del Estado de Durango
12:30-12:45	**De novo assembly of small RNA sequencing reads improves species assignment in combined parasite-host samples.** *Obed Ramírez Sánchez*. LANGEBIO CINVESTAV	**Characterization of catechol dioxygenases produced by aromatic hydrocarbons-degrading Pseudomonas strains obtained from the Gulf of México for its use in bioremediation.** *Selma Julieta Rodríguez-Salazar.* IBT UNAM	**Cooperativity in a dimeric enzyme is boosted by a novel mechanism.** *Jorge Ángel Marcos-Viquez.* Department of Biochemistry, School of Medicine, UNAM
12:45-13:00	**Inquiry Based Science Education (IBSE) as a tool for learning Molecular Biology. The experience in México.** *Viviana Escobar-Sánchez.* Facultad de Ciencias, UNAM	**Antimicrobial activity of redclaw crayfish *Cherax quadricarinatus* Pro-rich recombinant peptide.** *Gisela Ruvalcaba-Rodríguez*. Universidad Autónoma de Chihuahua	**Precise design of protein and DNA nanomaterials through the engineering of protein polymers and DNA binding domains.** *Armando Hernández-García*. Wageningen Univ.

11:00 – 11:30 Coffee break Foyer CC Azul Ixtapa

11:30 – 13:00 Technical Conferences
Zeus Room IV

Chair: *Guadalupe Gutiérrez Escobedo*
IPICyT

11:30 – 12:15 UNIPARTS
Edición de Genomas
Carlos Humberto Martínez Paniagua,
Gerente Soporte Científico

12:15 – 13:00 AGILENT TECH
Científico de Aplicaciones, Agilent Technologies México
Alejandro Xchel Rivera González

13:00 – 14:30 Lunch

Simultaneous Symposia 7
14:30 – 16:30

Zeus Room I

Simposio Hispano Mexicano
Epigenetic and Systems Biology

Chair: *Osbaldo Resendis*
Instituto Nacional de Medicina Genómica

14:30 – 15:00 Evolution of regulatory landscapes
José Luis Gómez-Skarmeta
Centro Andaluz de Biología del Desarrollo (CABD), Consejo Superior de Investigaciones Científicas. Universidad Pablo de Olavide. Sevilla España

15:00 – 15:30 Regulatory non-coding RNAs as pathogenic factors in neurodegenerative diseases: a functional genomics approach
Eulàlia Martí
Dpt. Biomedicine, University of Barcelona, Spain

15:30 – 16:00 A multiscale modeling approach to study the evolution of antibiotic resistance.
Ayari Fuentes Hernández
Centro de Ciencias Genómicas, UNAM

16:00 – 16:30 Gene expression regulation during Drosophila development
Kasia Oktaba
CINVESTAV Unidad Irapuato

Simultaneous Symposia 8
14:30 – 16:30

Zeus Room II

Neuroscience & Neurobiology

Chair: *Susana Castro Obregón*
Instituto de Fisiología Celular, UNAM

14:30 – 15:00 Glucocorticoids in the striatum modulate the consolidation and retrieval of emotional memory
Gina L. Quirarte, Norma Serafín, Cristina Siller-Pérez, Renata Ponce-Lina, Rogelio Pegueros Maldonado, and Roberto A. Prado-Alcalá
Departamento de Neurobiología Conductual y Cognitiva
Instituto de Neurobiología, UNAM

15:00 – 15:30 When the striatum initiates a movement who drives it?
Fatuel Tecuapetla, Joaquim, Alvez da Silva, Rui Costa & Edgar Hernandez Diaz
Instituto de Fisiologia Celular, UNAM

15:30 – 16:00 Optogenetic Control of appetite: Deciphering the neurons that promote overeating
Ranier Gutiérrez
Laboratory of Neurobiology of Appetite
Pharmacology Department CINVESTAV

16:00 – 16:30 Energy metabolism dysregulation during aging and its role in synaptic function and integrity
Clorinda Arias
Instituto de Investigaciones Biomédicas, UNAM

Simultaneous Symposia 9
14:30 – 16:30

Zeus Room III

Cultural

Chair: *Jorge Luis Folch*
CEIB UAEM

14:30 – 15:00 MEXICO & USA: Partners in Scientific Research. Are we doing enough? Are they doing enough?
Salomón Bartnicki-García
Department de Microbiología
Centro de Investigación Científica de Ensenada

15:00 – 15:30 Endothelial Luminal Membrane-Glycocalyx *Functionalities in Health and Disease*
Rafael Rubio
University of Virginia. Universidad Autónoma de San Luis Potosí.
Maureen Knabb, West Chester University

15:30 – 16:00 Scholarly publishing: a case for Open Access
Gabriel Gasque
PLOS Biology

16:00 – 16:30 Xochicalco: In the house of the flowers
Jorge Luis Folch Mallol
Centro de Investigación en Biotecnología.
Universidad Autónoma del Estado de Morelos

Zeus Room

16:30 – 17:30 **Plenary Lecture**

Recambio de las “Señoritas Neuronas": mecanismos y limitaciones

Arturo Álvarez-Buylla Roces

Department of Neurological Surgery. The Eli and Edythe Broad Center of Regeneration Medicine
Stem Cell Research
School of Medicine. University of California, San Francisco

Chair: *Clorinda Arias Álvarez*
Instituto de Investigaciones Biomédicas, UNAM

17:30 – 18:00 **Elevator talks**
Chosen from poster presentations

SB-25 Transcriptomic analysis during the interaction of *Clavibacter michiganensis* subsp. michiganensis with two tomato species. **Leonardo Isaac Pereyra-Bistraín**

SB-32 Genomic analyses on phytopathogenic nematodes. **Luis Ángel Xoca-Orozco**

B-66 Study of the putative nuclear localization signals (NLS) of the cationic channel TRPV4. **Susana Méndez-Gómez**

GR-84 Transcriptional network evolution underlying biofilm formation in *Candida maltose*. **Aníbal Uriel Reyes Mérida**

IP-27 Suramin Evokes Two Effects on Human P2X Receptors of Macrophages. **Eydie Mariela Vargas-Martínez**

IP-31 Exposure to an Enriched Environment Attenuates Mouse Experimental Colitis. **Tomás Villaseñor Toledo**

MH-81 Polymorphism detection in genes associated to isoniazid metabolism for dosage adjustment in San Luis Potosi. **Pettet-Ruiz Guillermo**

MV-50 Determination of factors that define the tropism of astrovirus in established cell lines. **Guzmán-Ruiz Leticia**

P-6 Generation of scientific and technological strategies with a multidisciplinary and interinstitutional approach to face the threat represented by exotic ambrosia beetles to the agricultural and forestry sectors of Mexico. **José A. Guerrero-Analco**

P-7 Factors that regulate SPATULA (SPT) expression in *Arabidopsis thaliana.* **Angela Guadalupe Juárez-Corona**

P-10 Identification of candidate genes regulated by ATX1 and involved in root development. **Selene Napsucialy Mendivil**

CB-1 Proteins implicated during the formation and degradation of Lipid droplets in *Ustilago maydis*. **Minerva Georgina Araiza Villanueva**

CB-2 Changes of Acrosomal pH During Human Sperm Capacitation. **Gabriela Carrasquel Martínez**

CB-5 Expression of Septins 1 and 2 during the Oogenesis of *Aedes aegypti*. **José Ángel Rubio Miranda**

CB-6 A predicted cargo adaptor, CNI, plays an important role in traffic of specific essential material for the growth of *Neurospora crassa*. **Luis Enrique Sastré-Velásquez**

18:00 – 19:00 **Having coffee with…..**
Round Tables for students. Chosen from oral presentations
Artemisa, Venus & Executive Rooms. First Floor CC Azul Ixtapa

18:00 – 18:30 Becas Humboldt para estancias postdoctorales y de investigación en Alemania
Susana Castro
Diana Room
First Floor CC Azul Ixtapa

18:00 – 20:00 Poster Session III
Ixtapa Room, Hotel Azul Ixtapa

SB	SYSTEMS BIOLOGY & BIOINFORMATICS II
B	BASIC BIOCHEMISTRY III
BT	BIOTECHNOLOGY III
GR	GENETICS, EPIGENETICS AND GENETIC REGULATION III
IP	IMMUNOLOGY & PARASITOLOGY II
MH	MEDICINE, HEALTH & NUTRITION III
MV	MICROBIOLOGY AND VIROLOGY III
CB	CELL BIOLOGY
P	BIOCHEMISTRY AND PLANT MOLECULAR BIOLOGY

THURSDAY

Thursday November 8, 2018

Zeus Room

8:30 – 9:30 **Plenary Lecture**

ABC Transporters in Nutrient Homeostasis and Pathogenesis

Heather W. Pinkett

Department of Molecular Biosciences
Northwestern University

Chair: *Mariana Peimbert*
Universidad Autónoma Metropolitana

Simultaneous Sessions

9:30 – 11:00	**19. Immunology & Parasitology Zeus Room I**	**20. Genetic, Epigenetic & Genetic Regulation IV Zeus Room II**	**21. Biochemistry and Plant Molecular Biology Zeus Room III**
	Chair: *Emma Saavedra* Instituto Nal. Cardiología	Chair: *Kasia Oktaba* CINVESTAV-Irapuato	Chair: *Carmen Quinto* IBT, UNAM
9:30-9:45	**Therapeutic model anti tumoral targeting E5 protein of human papillomavirus 16 to dendritic cells *in vivo*.** *Oscar Badillo-Godínez*. CISEI-INSP, SSA	***In vitro* development of splice-switching oligonucleotides to revert intronic DRD2 polymorphisms effect associated to Substance Use Disorders.** *Alejandro Bustos Cortés*. INMEGEN	**Maize root exudates. Large-scale phenotyping of a tropical and temperate diversity panels.** *Martha G. Lopez-Guerrero.* Department of Biochemistry, University of Nebraska-Lincoln
9:45-10:00	**Apoptosis of mouse hippocampal cells induced by *Taenia crassiceps* metacestode factor.** *Natalia Ivanovna Copitin Niconova*. Instituto de Fisiología Celular, UNAM	**Finding a 5-hydroxymethylcytosine "fingerprint" during hepatocyte differentiation. A clue for early cancer detection and intervention?.** *Jesús Rafael Rodríguez-Aguilera.* IFC, UNAM	**Low levels of polyamine alter resistance in Arabidopsis to necrotrophic and hemi-biotrophic pathogens.** *Ana Isabel Chávez Martínez.* División de Biología Molecular, IPICYT AC
10:00-10:15	**Searching for the natural ligand of the Human Aminopeptidase N (CD13).** *Georgina Ivette López-Cortés*. Instituto de Investigaciones Biomédicas, UNAM	**Participation of the epigenetic factor BORIS in transcriptional regulation of genes involved in ovarian cancer.** *Ernesto Soto-Reyes*, Natural Sciences Department, UAM	**Crosstalk between Methyl jasmonate and Polyamines on the Arabidopsis growth.** *Maria Azucena Ortega Amaro.* División de Biología Molecular, IPICYT AC
10:15-10:30	**Lipids induce the formation of germinal centers in the same time as a protein antigen.** *Claudia Albany Reséndiz Mora*. Escuela Nacional de Ciencias Biológicas – IPN	**Epigenetic modulation of Ca^{2+} homeostasis genes by Resveratrol helps to explain its anticancer properties.** *Ángel Zarain-Herzberg.* School of Medicine, UNAM	**The role of the TRPV channel in osmobiosis of the tardigrade *Hypsibius dujardini*.** *Erendira Anai De la Torre-Gonzalez*. Facultad de Ciencias Naturales, Universidad Autonoma de Queretaro
10:30-10:45	**A *Taenia crassiceps* factor induces apoptosis of spleen CD4+ T cells and TGF-β and Foxp3 gene expression in mice.** *Nadia Zepeda Córdova*. Instituto de Fisiología Celular, UNAM	**Long term effect of early overnutrition in the transcriptome of Wistar rat liver (*Rattus norvegicus*).** *Miguel Angel Espinoza Camacho.* National Institute of Genomic Medicine	**NaTrxh is an essential protein in the pollen rejection response in *Nicotiana*, which reduces to S-RNase increasing its activity.** *María Daniela Torres Rodríguez.* Facultad de Medicina, UNAM
10:45-11:00		**Comparison of microRNAs quantity in blood serum on patients from 65 years and older with frailty and patients clinically functional.** *García-Gamboa Ada Paulina*. Hospital General de México	**Characterization of the function of long non-coding RNA *lncTATA* on the development of *Arabidopsis thaliana*.** *Susana Isabel Vargas Camacho.* Facultad de Ciencias Químicas, UASLP

Thursday November 8, 2018

11:00 – 11:30 Coffee break Foyer CC Azul Ixtapa

Zeus Room

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11:30 – 12:30 **Plenary Lecture**

From genomics to levitating cells and rare diseases

Lars Steinmetz

EMBL Heidelberg/Stanford Genome Technology Center
Heidelberg, Germany

Chair: *Eugenio Mancera*
CINVESTAV Irapuato

13:00 – 14:30 Lunch

14:30 – 16:30 Poster Session IV
Ixtapa Room, Hotel Azul Ixtapa

B	BASIC BIOCHEMISTRY IV
BT	BIOTECHNOLOGY IV
GR	GENETICS, EPIGENETICS AND GENETIC REGULATION IV
MH	MEDICINE, HEALTH & NUTRITION IV
MV	MICROBIOLOGY & VIROLOGY IV
T	TOXICOLOGY II
ST	SIGNAL TRANSDUCTION II

Zeus Room

16:30 – 17:30 **Closing Lecture**

Therapeutic tRNA synthetase inhibition activates a novel arm of the amino acid response pathway

Whitman M, Keller T, Sundrud M, Yeo CY, Kim YJ, Zocco, D., Rao, A., Edenius,M., Zhou CQ

Department of Developmental Biology, Harvard School of Dental Medicine, Department of Cell Biology, Harvard Medical School

Chair: *Roberto Sánchez Olea*
Universidad Autónoma de San Luis Potosí

17:30 – 18:00 Final announcements and Closing Ceremony

21:00 Closing Dinner

POSTER SESSIONS

SYSTEMS BIOLOGY & BIOINFORMATICS I

SB-1 Proteomics and metabolomics for Southwest of Mexico from CICY**. Aguilar-Hernández V**. Unidad de Bioquímica y Biología Molecular de Plantas, CICY.

SB-2 Conformational changes regulate the half-life of proteins of the Bcl-2 family**. Luis Alberto Caro Gómez.** Bioquímica y Biofísica Computacional, ENMH, IPN

SB-3 Evaluation of the role of Spo0B as limiting factor in the information processing and decision making of the sporulation system of *B. subtilis.* **Nori Castañeda Gómez**.Instituto de Biotecnología, UNAM

SB-4 Viral metagenomic comparative analysis of the deep-sea hydrothermal vents. **Hugo G. Castelán Sánchez.** Instituto de Investigación en Ciencias Básicas y Aplicadas, UAEM

SB-5 Important ribosomal mutations are revealed by genomics of *Avibacterium paragallinarum.* **María Elena Cobos Justo**. Instituto de Ciencias. BUAP

SB-6 Secretome prediction of Two *M. tuberculosis* clinical isolates reveals their high antigenic density and potential drug targets. **Fernanda Cornejo-Granados**. Instituto de Biotecnología, UNAM

SB-7 The genetic interaction landscape of long lived mutants in *Saccharomyces cerevisiae*. **Erika Viridiana Cruz Bonilla**. Unidad de Genómica Avanzada. LANGEBIO. CINVESTAV

SB-8 Congenital absence of uterus: elucidation of the gene modules involved in the development of the female urogenital tract. **Fernando Fernández.** Unidad de Genética, Hospital General de México

SB-9 Functional analysis of genes expressed during cell differentiation of *Sclerotium cepivorum* Berk. **David Alberto García Estrada**. División de Ciencias Naturales y Exactas. Universidad de Guanajuato

SB-10 Identification of trans-acting riboswitches in bacterial genomes. **Edgar Rodríguez García.** Departamento de Microbiología Molecular. Instituto de Biotecnología – UNAM

SB-11 Behavior of the antimicrobial peptides Pin2 and Pin2GVG in membranes. Molecular Dynamics Simulations. **Ramón Garduño-Juárez**. Instituto de Ciencias Físicas, UNAM

SB-12 Characterization of the docking mechanism of peptide variants of discrepin on the Kv4.3 potassium channel. Molecular dynamics simulations. **Ramón Garduño-Juárez**. Instituto de Ciencias Físicas, UNAM

SB-13 Phylogenetic analysis of members of the MAP kinase family (MAPK) in Viridiplantae. **José Manuel González-Coronel**. Instituto de Biotecnología, UNAM

SB-14 A matrix-based stoichiometric model of *Helicobacter hepaticus* metabolism. **José I. Hernández-Oropeza**. Faculty of Engineering, Universidad La Salle México

SB-15 Cracking the mysteries of zygotic embryogenesis in avocado by systems biology. **Janet Juarez-Escobar.** Instituto de Ecología A.C

SB-16 Metabolic role of aldehyde dehydrogenases in Pseudomonas aeruginosa PAO1**. Adriana Julián-Sánchez**. Facultad de Medicina, UNAM

SB-17 Identification and analysis of the genes involved in the biosynthetic pathways of cellulose and lignin in *Agave tequilana* Weber. **Luis Fernando Maceda López.** Colegio de Postgraduados Campus Campeche

BASIC BIOCHEMISTRY I

B-1 Does SPC13, an antimicrobial activity found in the venom of the *scolopendra polymorpha*, also have histone H3 properties? **Carolina Abarca Camacho**. Centro de Investigación en Biotecnología. UAEM

B-2 Purification and characterization of the paralogous enzymes *Bat1* and *Bat2* of *Saccharomyces cerevisiae*. **Beatriz Aguirre López**. Department of Biochemistry and Structural Biology, Instituto de Fisiología Celular. UNAM

B-3 The role of renal betaine aldehyde dehydrogenase in the carnitine biosynthesis. **José Alberto Alcantar-Ibarra.** Ciencias Químico-Biológicas y Agropecuarias, Universidad de Sonora Unidad Regional Sur

B-4 Development of a computational algorithm for designing inhibitors under a fragment-based approach. **María Fernanda Álvarez Esquinca**. Departamento de Bioquímica y Biología Estrucural, Instituto de Fisiología Celular. UNAM

B-5 RNA Aptamer selection to *Rickettsia rickettsia*. **Ana Mercedes Andrade Aguilar.** Facultad de Medicina. Universidad Autónoma de Baja California

B-6 The regulation of metabolic syndrome and hepatic damage in an experimental model by an adenosine derivated**. Alejandro Rusbel Aparicio-Cadena**. Instituto de Fisiología Celular, UNAM

B-7 Validation of biomarkers for the detection of *Helicobacter pylori*-associated gastric cancer. **Arenas Linares Ana Silvia.** Universidad Autónoma del Estado de Morelos

B-8 Characterization of the reaction center spectrum of *Rhodovibrio salinarum*. **Andrés Arenas Navarro**. Department of Molecular Genetics, Institute of Cellular Physiology. UNAM

B-9 Construction of an endoxifen binding protein with high affinity and selectivity. **Emma Liliana Arévalo-Salina**. Ingeniería Celular y Biocatálisis, Instituto de Biotecnología, UNAM

B-10 Modulating hydrolysis/transfer function through the analysis of inter-residue protein contacts in the alpha-amylase enzyme family. **Rodrigo A. Arreola-Barroso**. Instituto de Biotecnología, UNAM

B-11 *Phaseolus vulgaris* rhizosphere stimulates *Rhizobium* conjugation: home, food and love? **Luis Alfredo Bañuelos-Vázquez**. Ingeniería Genómica. Centro de Ciencias Genómicas, UNAM

B-12 Effect of glucose concentration on the activity of ubiquitin E3 ligase of Mdm2. **Raul Barzalobre Geronimo**. Investigacion Médica en Bioquimica. Centro Medico Nacional Siglo XXI. IMSS

B-13 Participation of residue E261 in the structural stability of the glucose-6-phosphate dehydrogenase from *Pseudomonas aeruginosa***. Edaena Benítez-Rangel.** Facultad de Estudios Superiores Iztacala, UNAM

B-14 Differential gene expression analysis between *Heliopsis longipes* and *Heliopsis annua* to identify genes related to Alkamides Biosynthesis. ***Génesis V. Buitimea-Cantúa.*** CINVESTAV Irapuato

B-15 The amino-terminal region of Pet309 is important to interact with its Mrna target and with mitoribosome. **Yolanda Camacho-Villasana**. Departamento de Genética Molecular, Instituto de Fisiología Celular. UNAM

B-16 Chemical and biological analysis of methanolic extract from pomegranate (*Punica granatum* L) leaves with antibacterial activity against *Ralstonia solanacearum*. **Manuel Carrillo-Morales**. Universidad Politécnica del Estado de Morelos

B-17 GLP-1r Activation Decreases HIF-1A Expression in the Kidney after Ischemia Reperfusion in Obese Rats. **Susana Del Carmen Castro-Meza**. Ciencias Químico-Biológicas y Agropecuarias. Universidad de Sonora

B-18 Characterization of gluconeogenic activity in Intermittent Fasting. **Silvia Cristina Cervantes Gutiérrez**. Institute of Neurobiology. National Autonomous University of Mexico

B-19 Ligand based pharmacophore modeling and virtual screening to find new benzimidazole derivatives as potential PTP1B inhibitors. **Alondra Sarahi Chaidez Avila**. Facultad de Medicina y Nutrición, Universidad Juárez del Estado de Durango

B-20 Effect of IFC-305 on autophagy in the sequential model of CIRRHOSIS-HCC and in vitro. **Chávez E**. Instituto de Fisiología Celular, UNAM

B-21 The role of oxidative stress and gender in the erythrocyte arginine metabolism and ammonia management in patients with TYPE 2 diabetes. **Martha L. Contreras-Zentella.** Biología Celular y Desarrollo. Instituto de Fisiología Celular, UNAM

B-22 Cloning and biochemical characterization of three Glucose-6-Phosphate Dehydrogenase mutants presents in the Mexican population. **Yadira Yazmín Cortés-Morales.** Instituto Nacional de Pediatría. SS.

B-23 NaStEP stability in pollen tubes accounts for self-incompatibility in *Nicotiana alata*. **Yuridia Cruz-González Zamora.** Facultad de Química, UNAM

B-24 Effect of heavy metals on *Ustilago maydis* respirasome. **Jaime Abraham de Lira Sánchez**. Departamento de Bioquímica. Facultad de Medicina, UNAM

B-25 C-terminal amidation - Identification and expression of the amidating enzyme PAM from Scorpion *Centruroides noxius*. **Gustavo Delgado Prudencio.** Departmento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología, UNAM

B-26 Isolation and characterization of the N66 protein from the mother oyster pearl shell *Pinctada mazatlanica***. Rosa Virginia Dominguez Beltran**. Laboratorio de Genética Molecular. CIBNOR

B-27 Biosynthesis of ATP in mitochondria from skeletal and cardiac muscle: Effect of metabolic syndrome and aging. **Mohammed El-Hafidi**. Instituto Nacional de Cardiología “Ignacio Chávez”

B-28 Characterization of the triosephosphate isomerase from *Encephalitozoon intestinalis*. Proposal of a new pharmacological target. **Sergio Enríquez Flores.** Instituto Nacional de Pediatría

B-29 The conformational landscape of proteins: study of de novo designed TIM barrels. **Sergio Romero-Romero**. Facultad de Medicina, UNAM

BIOTECHNOLOGY I

BT-1 Expression of single chain variable fragment scFv 6009F in *Pichia pastoris*. **Mariel Adame Román.** Centro de investigación en Biotecnología. UAEM

BT-2 Permutations of the loops of Ts1 by those of the neurotoxin CssII: in vitro folding and specificity on voltage-dependent sodium channels. **Germán Obed Aguilar Carlos.** Instituto de Biotecnología, UNAM

BT-3 Characterization of extracellular enzymatic extract obtained from *Phlebia floridensis* strain isolated from Yucatan Peninsula with a novel peroxidase and oxidase profile. **Roberto Amezquita-Novelo.** Tecnológico Nacional de México

BT-4 Analysis of cellulases produced by microorganisms isolated from sites contaminated by heavy metals. **Alondra Andrade González**. Unidad Profesional Interdisciplinaria de Ingeniería campus Guanajuato. IPN

BT-5 Stabilization of Cytochrome P450 from *Bacillus megaterium*, through changes in positions C774A and C1000A in its Reductase domain. **Manuel Alejandro Arévalo Salina.** Universidad Autónoma Benito Juárez de Oaxaca

BT-6 Purification and characterization of the extracellular laccase of Didymosphaeria sp. (syn.= *Paraconiothyrium brasiliense* sensu lato). **Marina Arredondo-Santoyo.** Facultad de Medicina Veterinaria y Zootecnia, Universidad Michoacana

BT-7 Identification and characterization of the biotechnological potential of a wild strain of Didymosphaeria sp. (syn.= *Paraconiothyrium brasiliense* sensu lato). **Marina Arredondo-Santoyo**. Universidad Michoacana

BT-8 Characterization of the protein extract of the *Bromelia karatas* fruit and its antimicrobial activity against *Candida albicans*, *Salmonella typhimurium* and *Listeria monocytogenes*. **Elva Avalos-Flores.** Tecnológico Nacional de México

BT-9 Redox metabolites production of *Pseudomonas aeruginosa* NEJ01R using biodiesel process wastewaters. **Francisco Javier, Bacame-Valenzuela**. Centro de Investigación y Desarrollo Tecnológico en Electroquímica, S.C

BT-10 Fungi molecular identification of Tar Spot Complex on corn. **Rosibel Bahena Oregón.** Universidad Autónoma de Guerrero

BT-11 Overexpression of dehydrin DHN1 and rubisco activase in maize plants transformed by the intragenic method. **Raymundo Belmont Valadez.** Departamento de Bioquímica, Facultad de Química, UNAM

BT-12 Development of a recombinant protein GroEL of *B. canis*, for use as a diagnostic antigen for canine brucelosis. **Nancy Belem Beltrán Maldonado.** Faculty of Veterinary Medicine and Zootechnics

BT-13 Removal of the Synthetic Dye Remazol Brilliant Blue R by Bioadsortion on Biomass from Aspergillus spp. **Irma Bernal Lugo**. Facultad de Química, UNAM

BT-14 Neuronal localization assays of Rapamycin-loaded PLGA nanoparticles for potential treatment of Spinocerebellar Ataxia type 7. **Fabiola Vianet Borbolla-**

Jiménez. Instituto Nacional de Rehabilitación “Luis Guillermo Ibarra Ibarra”

BT-15 *Bacillus thuringiensis* as: growth factor and bioinsecticide helped by a synergistic protein. **Martha Cecilia Bravo Rivas.** Division Life Sciences, University of Guanajuato

BT-16 Development of monitoring oxygen and carbon dioxide transfer rate (OTR and CTR) device and cotton closure simulation during *Pichia pastoris* shake flask cultures. **Daniel Cabrera Santos**. Instituto de Investigaciones Biomédicas, UNAM

BT-17 Extraction and chemical and functional characterization of squalene, starch and protein of *Amaranthus* species. **Oscar de Jesús Calva Cruz.** División de Biología Molecular. Instituto Potosino de Investigación Científica y Tecnológica A.C.

BT-18 Influence of *SNF1* gene on glycolytic flux addressed to aerobic fermentation (Crabtree effect) of *Saccharomyces cerevisiae*. **Andres Carrillo-Garmendia**. Instituto Tecnológico Superior de Ciudad Hidalgo

BT-19 Morphological study of dedifferentiation process on *Rosmarinus officinalis* leaf explants by digital image analysis. **Yessica Casales-Tlatilpa**. Centro de Desarrollo de Productos Bióticos. IPN

BT-20 Characterization chromatography of the avocado seed. **Dolores Castañeda-Antonio**. Instituto de Ciencias, CICM. BUAP

BT-21 Citric acid effect in fungal tolerance to chromium stress. **Oscar-Guadalupe Castillo-Martínez.** Instituto Tecnológico Superior de Irapuato

BT-22 Neutralizing capacity of a hyperimmune sera from *Crotalus durissus terrificus* snake venom conjugates. **Irene Maria Castillo Perez.** Instituto de Biotecnologia, UNAM

BT-23 Cofactor specificity engineering of a Water-Forming NADH oxidase from *Giardia lamblia* as Universal Regenerating System for Redox Reactions. **Adriana Castillo Villanueva**. Laboratorio de Bioquímica-Genética. Instituto Nacional de Pediatría

BT-24 Composite coating of AgNP'S with antimicrobial activity. **Berenice Castro Rodriguez**. Centro de Investigación y Desarrollo Tecnológico en Electroquímica

BT-25 Secondary metabolites from Metarhizium with effect on bacteria. **Karla Yadira Cervantes-Quintero**. Departamento de Biología. División de Ciencias Naturales y Exactas, Universidad de Guanajuato

BT-26 Silencing and overexpression of the aquaporin gene pvpip 2-4 affect nodulation in *Phaseolus vulgaris*. **Mariana Esther Cesario Solis**. Biología Molecular de Plantas. Instituto de Biotecnología, UNAM

BT-27 Isolation of bacteriophages for the treatment of *Pseudomonas aeruginosa* infections. **María I. Chávez Béjar**. Universidad Politécnica del Estado de Morelos

BT-28 Detection of intestinal bacteria in drinking water and residuals. **Lorena Chávez González.** Unidad de Microarreglos de DNA, Instituto de Fisiología Celular, UNAM

BT-29 Molecular characterization of *Pycnoporus* spp. **Lizbeth Coronel Pastor.** Centro de Investigación en Biotecnología, UAEM

BT-30 Genetic characterization and selection of rhizobacteria from *Agave americana* L for use as biofertilizers. **Néstor Hugo Cruz Pérez.** Instituto Tecnológico de Tuxtla Gutiérrez

BT-31 Expresión, purificación y caracterización fisicoquímica y estructural del dominio catalítico de la MMP-16, potencial blanco terapéutico contra Aterosclerosis y Cáncer. **Armando Cruz-Rangel.** Instituto Nacional de Medicina Genómica

BT-32 Hydrocarbon degrading activity of a bacterial consortium isolated from sediments of the Gulf of Mexico. **Diego Humberto Cuervo Amaya**. Instituto de Biotecnología, UNAM

BT-33 Obtaining 3-hydroxyanthranilic acid from the liquid culture of *Pycnoporus cinnabarinus* for the synthesis of antibiotics. **Elizabeth Cuevas-Reyes.** Biotechnology Research Cente

BT-34 Protective effect of acetonic extract of leaves of *Ficus* sp on oxidative damage in HaCaT cells. **Brenda de la Cruz Concepción.** Faculty of Biological Chemistry Sciences. Autonomous University of Guerrero

BT-35 Prospecting and characterization of psychrophiles microorganisms plant growth promoters. **Irán Tapia Vázquez.** Centro de Investigación en Biotecnología. UAEM

GENETICS, EPIGENETICS AND GENETIC REGULATION I

GR-1 Protein dosage of the lldPRD operon depends on processing of the primary transcript. **Lidia Esmeralda Angel Lerma**. Genética Molecular, Instituto de Fisiología Celular. UNAM

GR-2 Transcriptomic landscape of the radioresistance in breast cancer. **Elena Aréchaga Ocampo**. Metropolitan Autonomous University, Cuajimalpa Unit.

GR-3 Advances in the molecular study of the musculoskeletal system in tropical gar (*Atractosteus tropicus*). **José Natividad Arias Jiménez**. Universidad Juárez Autónoma de Tabasco

GR-4 Chia seeds extract modify the expression of *Nos3* and *Bdkrb2* genes in hypertensive rats. **Gerardo Ismael Arredondo-Mendoza**. Centro de Investigación en Nutrición y Salud Pública. Universidad Autónoma de Nuevo León

GR-5 Minigenes as reporter systems for functional genomics. **Elizabeth Cavazos Benhumea**. Instituto Nacional de Medicina Genómica

GR-6 Study of Mitochondrial DNA in Modern Nahuas from Central Mexico: Overview of the Genetic Relationships. **Víctor Hugo Avilés Chávez.** Departamento de Biología Celular, Facultad de Ciencias, UNAM

GR-7 Effect of epigenetic drugs on regulation of *c-MYC* promoter in hepatic cell lines. **Paulina Janeth Barraza Reyna.** Centro de Investigación Biomédica del Noreste. Instituto Mexicano del Seguro Social

GR-8 Impact of high fat diet on the intranuclear location of the circadian gene *Dbp*. **Fernando Becerril Pérez.** Instituto de Investigaciones Biomédicas, UNAM

GR-9 HIF-1 and VEGF gene expression by valproate in human hepatome cells. **Mario Bermúdez de León.** Vicerrectoría de Ciencias de la Salud, Universidad de Monterrey. Centro de Investigación Biomédica del Noreste. IMSS

GR-10 Metabolic regulation of β-NAC subunits in the yeast *Saccharomyces cerevisiae*. **José Ernesto Bravo Arévalo.** Departamento de Genética Molecular, Instituto de Fisiología Celular, UNAM

GR-11 DNA methylation alterations in adipocytes derived from obese diabetic patients. **Federico Centeno Cruz.** Inmunogenómica y Enfermedades Metabólicas. Instituto Nacional de Medicina Genómica

GR-12 Characterization of the microRNA-137 in the breast cancer cell line MDA-MB-231. **Andrea Viridiana Cervantes Ayala.** Hospital de Pediatría "Silvestre Frenk Freund", CMN Siglo XXI, IMSS

GR-13 Intra- and inter-plasmid regulation of conjugative transfer in *Sinorhizobium fredii* strain GR64. **Laura Cervantes-De la Luz.** Centro de Ciencias Genómicas, UNAM

GR-14 Use of CRISPR-Cas9 to edit the promoter of the microRNA effector protein. **Diana Chavira Desales**. Instituto Nacional de Medicina Genómica

GR-15 Functional effect of variants with uncertain clinical significance (VUS) of the BRCA1 / BRCA2 genes in Mexican women. **Manuel Misael Coca-González.** Facultad de Estudios Superiores Iztacala. UNAM

GR-16 Methylation profile distinguishes different outcomes in patients with locally advanced cervix cancer. **Carlos Manuel Contreras-Romero.** Instituto Nacional de Cancerología

GR-17 Characterization of mRNA methylation (m6A) in response to extracellular stimulus. **Perla Diana Coronado Monroy.** Instituto Nacional de Medicina Genómica

GR-18 mir- 26a, epigenetic regulator associated with colorectal cáncer. **Jossimar Coronel Hernández.** Facultad de Estudios Superiores Iztacala. UNAM

GR-19 Evaluation of the expression level of miR-132, -203 and -212 in breast cancer tissue and its possible role as post-transcriptional regulators of BRCA-1, BRCA-2 and ATM. **Pablo Cortés Pérez.** Instituto Nacional de Cancerología

GR-20 Characterization of epigenetic changes associated with the metabolic memory In vitro. **Martí David Wilson Verdugo**. Departamento de Biología Celular y del Desarrollo. Instituto de Fisiología Celular, UNAM

GR-21 Second-site suppressors of mutations in essential genes RHE_PE00001 and RHE_PE00024 of *Rhizobium etli* CFN42 support a role in peptidoglycan synthesis. **Araceli Dávalos.** Centro de Ciencias Genómicas UNAM

GR-22 Relationship between chromatin structure and gene expression linked to stress response in *Debaryomyces hansenii*. **Ileana de la Fuente Colmenares.** Laboratorio de Biología Molecular y Genómica, Facultad de Ciencias, UNAM

GR-23 Estradiol effects on EZH2 expression in human glioblastoma cells. **Aylin Del Moral-Morales.** Unidad de investigación en Reproducción Humana. Instituto Nacional de Perinatología. Facultad de Química, UNAM

GR-24 Rhizobium genomic edition using the CRISPR / Cas9 system. **Rafael Díaz Méndez.** Programa de Ingeniería Genómica, Centro de Ciencias Genómicas, UNAM

GR-25 Negative Regulation of Serine Threonine Kinase 11 (STK11) through miR-100 in head and neck cancer. **Gabriela Figueroa González.** Laboratorio de Genómica. Instituto Nacional de Cancerología

GR-26 Role of Transcription Factor NusG in the Adaptive Mutagenesis of *Bacillus subtilis*. **Guillermo de Jesús Flores Álvarez,** Universidad de Guanajuato

GR-27 Evaluation of MMP2, Notch-1 and Snail1 in siHa cells transfected with members of the miR-34 family. **Ixamail Fraire Soto.** Laboratorio de microRNAs, Unidad Académica de Ciencias Biológicas, Universidad Autónoma de Zacatecas

GR-28 High glucose-diet reduces *C. elegans* longevity by autophagy in an HLH-30/TFEB dependent manner. **Berenice Franco-Juárez.** Unidad de Genética de la Nutrición. Instituto de Investigaciones Biomédicas UNAM - Instituto Nacional de Pediatría

GR-29 Stress induced expression of *SLM35* in *Saccharomyces cerevisiae*. **Hernán Romo-Casanueva**. Departamento de Genética Molecular, Instituto de Fisiología Celular, UNAM

IMMUNOLOGY & PARASITOLOGY I

IP-1 Evaluation of enzymes of clinical relevance in serum and hepatic tissue after abdominal surgery in rats. **Violeta Aburto Luna.** Facultad de Ciencias Químicas. BUAP

IP-2 Genetic characterization of T cell and B cell receptors of California sea lion. **Aranzazu Arias-Rojas**. Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro

IP-3 Study of the death induced by *E. histolytica* trophozoites to different cell lines and its correlation with amebopore. **Barrón Oscoy Yurubi**. Research Unit in Experimental Medicine, Medicine Faculty UNAM

IP-4 Low density neutrophils from healthy donors display an activated phenotype. **José Carlos Blanco Camarillo.** Instituto de investigaciones Biomédicas, UNAM

IP-5 Molecular characterization of a rich cysteine protein from *Anopheles albimanus*: its possible involvement during the mosquito immune response. **Victor M. J. Cardoso-Jaime**. Centro de Investigación Sobre Enfermedades Infecciosas INSP

IP-6 Levels of 17β-oestradiol and testosterone during the infection with *P. berghei* ANKA. **Luis Antonio Cervantes-Candelas**. Laboratorio de Inmunología Molecular, Facultad de Estudios Superiores Zaragoza, UNAM

IP-7 Effect of *Cymbopogon citratus* and *Artemisia mexicana* on the expression of IL1β in mice infected with *P. berghei* ANKA. **López-Padilla MS**. Laboratorio de Inmunología Molecular, Facultad de Estudios Superiores Zaragoza, UNAM

IP-8 Analysis of the expression of CD163 and TWEAK in macrophages and dendritic cells of patients with acute myocardial infarction and its correlation with cardiac function. **Laura Sherell Marín Jauregui**. CICSaB, Facultad de Medicina, UASLP

IP-9 Insight about the lethal effect of curcumin on *Taenia crassiceps* cysticerci. **José de Jesús Martínez-González**. Departamento de Bioquímica, Facultad de Medicina, UNAM

IP-10 Anti-*Trichomonas vaginalis* effect of tritrpticin-derived peptides. **Moisés Adrián Martínez-Padilla**. Departamento de Biología.Universidad de Guanajuato

IP-11 The thioredoxin reductase from *Entamoeba histolytica* as a promising target against amoebiasis disease. **Yoalli Martínez Pérez**. Unidad de Investigación en Medicina Experimental, Facultad de Medicina, UNAM

IP-12 Cell cycle characterization in the *Anopheles albimanus* midgut cells. **Krystal Maya Maldonado**. Center for Research and Advanced Studies of the National Polytechnic Institute

IP-13 Cloning, purification and biochemical characterization of the recombinant Iron-Sulfur Flavoprotein of *Entamoeba histolytica*. **Paulina Mejía Del Castillo**. Facultad de Medicina, UNAM

IP-14 Fecal Lactobacilli Count in BALB/c Mice Treated with Bovine Lactoferrin. **Melendez-Avalos Araceli**. Depto. Sistemas Biológicos, UAM-Xochimilco

IP-15 Anti-*Trichomonas vaginalis* activity of hexanoic extract of avocado seed. **Blanca Yessenia Mendez-Grimaldo**. Universidad de Guanajuato

IP-16 Recombinant protein harboring relevant epitopes from Influenza A H1N1, as a new candidate vaccine against flu. **Karen Lizbeth Reyes-Barrera**. División de Biología Molecular, IPICYT

MEDICINE HEALTH & NUTRITION I

MH-1 Study of calcium signaling generated by activation of a photosensitizing substance and its propagation in prostate cancer cells. **María Coral Aguilar Santos**. Instituto de Fisiología. Benemérita Universidad Autónoma de Puebla

MH-2 Expression of the transcription factors HIF1a, HIF2a and HIF3a in pulmonary fibroblasts exposed to hypoxia. **Arnoldo Aquino-Gálvez**. National Institute of Respiratory Diseases "Ismael Cosío Villegas"

MH-3 Participation of the STIM1 / Orai1 complex in cell proliferation and migration, induced by epidermal growth factor (EGF) in the MDA-MB 231 cell line. **Avalos-López Juan Manuel**. Instituto de Fisiología, BUAP

MH-4 Salivary MMP-2 activity in Type 2 Diabetes Mellitus patients with Periodontitis, **Brenda Ayerdi Nájera**. Unidad de Innovación Clínica y Epidemiológica del Estado de Guerrero

MH-5 Changes of urokinase-type plasminogen activator in breast cancer cells in presence of nicotinamide. **María Guadalupe Martínez Hernández**. Morphology and Function Unity, Faculty of Professional Studies Iztacala, UNAM

MH-6 Identification of new mutations in *DYRK1B* associated with monogenic metabolic syndrome. **Francisco Barajas-Olmos**. Instituto Nacional de Medicina Genómica

MH-7 ER-β activation regulates expression of VEGF-C / VEGFR-3 complex in triple negative breast cancer cells. **Zuleyma Basilio Lino**. Instituto de Fisiología, BUAP

MH-8 Immunophenotypic characterization of GMP-grade mesenchymal stem cells derived from human adipose tissue. **Mayra Y. Bazán-Álvarez**. Departamento de Bioquímica. Facultad de Medicina, UANL

MH-9 Long-term analysis of the electrocardiogram as a method of evaluation of the development of hypertrophy in conscious rats with spontaneous hypertension. **Bocanegra Alfaro Ana Karina**. Institute of Physiology, BUAP

MH-10 Participation of the heat shock protein 27 in the regulation of the death of chondrocytes in the articular cartilage of an experimental model of osteoarthritis. **Moisés Cabrera-González**. CINVESTAV-IPN

MH-11 Effect of the intranasal vaccine HB-ATV-8 on atherogenesis and non-alcoholic fatty liver disease in a rabbit model of atherosclerosis. **Sandra Calixto Tlacomulco**. Instituto de Fisiología Celular, UNAM

MH-12 Frequency of expression of TGF-βRII and its association with sex steroid hormone receptors in ovarian serous carcinomas. **Argelia Calvillo Robledo**. Universidad Autónoma Metropolitana

MH-13 Evaluation of the fibroine and the alginate as a biomaterial of scaffolding for the growth of cells from the vascular estromal fraction. **Susana G. Cardenas-Ramos**. Facultad de Medicina. Universidad Autonoma de Nuevo Leon

MH-14 Expression profile and subcellular localization of the Heat Shock Proteins, Hsp90α and Hsp90β, with the androgen receptor, allows to identify a patients subgroup with prostate cancer susceptible to the Hsp90 inhibition in primary cell cultures. **Rafael Castillo Negrete**. UNAM. Instituto Nacional de Cancerología

MH-15 Expressions of metalloproteinases associated with p-ERK in ovarian tumors. **Castillo Sánchez Rocío**. Facultad de Medicina, UNAM

MH-16 Biological implications of the proteins and microRNAs contained in hepatocellular carcinoma cells-derived extracellular vesicles. **Luis Alberto Castro Sánchez**. Centro Universitario de Investigaciones Biomédicas, Universidad de Colima

MH-17 Effect of acetonic extract of *Ficus* spp. leaves on the migration capacity of tumour cells MDA-MB-23. **Lorena Cayetano Salazar**. Autonomous University of Guerrero

MH-18 The expression the receptor of PRL and GH is regulated by the activation of estrogen receptor β (ER-β) of triple negative breast cancer cells. **Cecilia Cruz Neria**. Instituto de Fisiología, BUAP

MH-19 Proteomic analysis of serum patients with insulin resistance. **Walter David Cruz Pineda**, Laboratory of Obesity and diabetes Research, Faculty of Chemical and Biological Sciences, University Autonomous of Guerrero

MH-20 Protein levels of pIRE1, ATF6, pPERK and BiP (Unfolded Protein Response mediators) in keratoconus. **Francisco Emmanuel Cruz Trápala.** Centro de Ciencias de la Salud, Universidad Autónoma de Aguascalientes

MH-21 Differential expression of proteins in an atypical presentation of Autoimmune Lymphoproliferative Syndrome. **Dulce Maria del Carmen Delgadillo-Álvarez**. Laboratorio Nacional de Servicios Experimentales. CINVESTAV

MH-22 Infections in Pediatric Patients with Neoplasia and Neutropenia of the Instituto Estatal de Cancerología “Dr. Arturo Beltrán Ortega” Acapulco, Guerrero. **Sacnite del Mar Díaz González**. Instituto Estatal de Cancerología “Dr. Arturo Beltrán Ortega”

MH-23 Atorvastatin and Rosuvastatin: undesirable side effects of high doses in hypercholesterolemic rodents. **Juan Cuauhtémoc Díaz Zagoya**. División de Investigación, Facultad de Medicina, UNAM

MH-24 Hypoglycemic effect of the aqueous extract of Galeana leaves (*Spathodea campanulata*) in diabetic rats. **María del Socorro Escamilla Barrera**. Faculty of Medicine and Biological Sciences “Dr. Ignacio Chávez”, Michoacán University

MH-25 Effect of *Spirulina maxima* in rats treated with fried oil as prooxidant. **María Teresa Espinosa García**. Depto. de Bioquímica, Facultad de Medicina, UNAM

MH-26 TLR4 activation contributes to mesenchymal phenotype in U87 cells, favoring their metastatic potential. **Miriam Fernández-Gallardo**. CINVESTAV-IPN

MH-27 PaDef defensin from avocado (*Persea americana* var. drymifolia) is cytotoxic to K562 chronic myeloid leukemia cells through extrinsic apoptosis. **Luis Jose Flores-Alvarez**. Facultad de Medicina Veterinaria y Zootecnia, Universidad Michoacana

MH-28 Differential expression profile of heat shock proteins of 90kDa, Hsp90α and Hsp90β, during the formation of 3D spheroids of hormone-sensitive and hormone-resistant prostate cancer cells. **Ilse Mariana Flores Hernández**. Instituto de Investigaciones Biomédicas, UNAM and Instituto Nacional de Cancerología

MICROBIOLOGY & VIROLOGY I

MV-1 Phylogenetic characterization of the genus Tobamovirus in children younger than six months of age. **Yarenci Aguado.** Instituto de Biotecnología, UNAM

MV-2 Isolation and purification of *E.coli* DH5α bacteriophages in wastewater and river water samples from Nuevo Leon, Mexico. **Anaí Amaral García**, Universidad Autónoma de Nuevo León

MV-3 Participation of the α-1 and α-2 helixes in the specificity of the toxin Cyt1Aa from *Bacillus thuringiensis* subsp. *Israelensis.* **Paulina Anaya Cárdenas.** Instituto de Biotecnología, UNAM

MV-4 The genetic variability of E7 HPV16 oncoprotein in cervical samples of woman from State of Guerrero. **Ramón Antaño Arias**. Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Guerrero

MV-5 Marine bacterial diversity with high potential to degrade pyrene isolated from Rosarito in Baja California**. Cynthia Lizzeth Araujo-Palomares.** Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California

MV-6 Functional analysis of Rep and CP promoters of a natural mutant of Tomato yellow leaf curl virus (TYLCV). **Jesús Aarón Avalos-Calleros**. División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica

MV-7 The role of microRNA-927 during the persistent infection with DENV serotype 2 in C6/36 mosquito cells. **Avila-Bonilla R. G.** Escuela Nacional de Medicina y Homeopatía, IPN

MV-8 The tyrR gene encoding the transcriptional regulator TyrR from *Azospirillum brasilense* Sp7 is involved in catabolism of alanine. **Beatriz Eugenia Baca**. Centro de Investigaciones en Ciencias Microbiológicas, BUAP

MV-9 Characterization of the response of *Arabidopsis thaliana* in interaction with auxin-producing rhizobacteria isolated from Chihuahuan Desert plants. **Fernando Balderas Hernández**. Universidad Juárez del Estado de Durango

MV-10 Characterization of a putative component of the Fla 2 flagellar system of *Rhodobacter sphaeroides*. **Teresa Ballado**. Instituto de Fisiología Celular, UNAM

MV-11 Determination of the regulatory network of quorum sensing in *Rhizobium leguminosarum* biovar viciae 248. **Lorena Y. Balón-Rosas.** Centro de Ciencias Genómicas, UNAM

MV-12 Stress study in *Bacillus subtilis* by use of artificial ribo-regulators. **Diana Barceló-Antemate.** Universidad Autónoma del Estado de Morelos

MV-13 Study of the regulation of PHB depolymerization in *Azotobacter vinelandii*. **Thalía Barrientos Millán**. Instituto de Biotecnología. UNAM

MV-14 Characterization of chaperonine HpGroEL of *Helicobacter pylori*. **Selene Becerril-Huesca**. Universidad Autónoma de la Ciudad de México

MV-15 Influence of foreign bus passengers in the composition of the microbiome of Mexico City subway. **Ana Luisa Bravo**. UAM Unidad Cuajimalpa

MV-16 Inhibitory effect of the nanoparticled compound Nbelyax™ over viral and fungal respiratory pahogens. **Carlos Cabello Gutiérrez**. Instituto Nacional de Enfermedades Respiratorias "Ismael Cosío Villegas"

MV-17 Cellular organization of the entomopathogenic fungus *Metarhizium anisopliae*. **Olga Alicia Callejas-Negrete**. Centro de Investigación Científica y de Educación Superior de Ensenada

MV-18 Surveillance of venezuelan equine encephalitis using cattle as sentinels in the Eastern Mexico-US transboundary region. **Castillo-Cervantes K.** FMVZ, UNAM

MV-19 *In vitro* antagonistic activity of bacteria isolated from marine ecosystems against *Vibrio parahaemolyticus*, causal agent of Acute Hepatopancreatic Necrosis Disease (AHPND) in *Litopenaeus vannamei* cultures. **Gabriel Enrique Cázares Jaramillo**. Facultad de Ciencias Biológicas. UANL

MV-20 Characterization of Merlin, a regulator of actin dynamics, in Aedes spp., mosquito vector of arboviruses. **Febe Elena Cázares-Raga.** Departamento de Infectómica y Patogénesis Molecular, CINVESTAV-IPN

MV-21 Characterization of bacteria associated with the rhizosphere of amaranth from a crop with a forest matrix in Tochimilsolco, Puebla. **Verónica Cepeda Cornejo**.Facultad de Ciencias Biológicas, BUAP

MV-22 The Abundance of bacteriophage CrAssphage in Mexican childrens with obesity it is related with enteric bacteria of gut microbiota. **Melany J. Cervantes Echeverria.** Departamento de Microbiología Molecular, Instituto de Biotecnología, UNAM

MV-23 Comparative study between diarrhoegenic *Escherichia coli* virotypes of the México City with virotypes of San Luis Potosí. **Ruth Adriana Cervantes-Olmos**. Facultad de Medicina, UNAM

MV-24 Molecular characterization, phylogenetic analysis and replication of a new begomovirus infecting bean in Nayarit, Mexico. **Martínez-Marrero Nadia.** División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica

SIGNAL TRANSDUCTION I

ST-1 Fc gamma RIIIb induces extracellular calcium influx without mobilization of calcium from intracellular deposits, in human neutrophils. **Omar Rafael Alemán**. Instituto de Investigaciones Biomédicas. UNAM

ST-2 Role of p53 and mutants (R175H and R273H) over canonical pathway of Wnt on a model of Cancer Stem Cells. **Eduardo Alvarado Ortiz**. Unidad de Investigación Biomédica en Cáncer, Instituto de Investigaciones Biomédicas, UNAM

ST-3 Mechanisms of secretion of the TGF-β cytokine from melanoma cells and its actions on mast cells. **Isabel Anaya Rubio**. Departamento de Biología Celular y Desarrollo, Instituto de Fisiología Celular, UNAM

ST-4 Heterologous calcium-dependent inactivation of Orai by neighboring TRPV1 channels modulates cell migration and wound healing. **Carlos Ernesto Bastian-Eugenio**. Instituto de Fisiología Celular, UNAM

ST-5 Evaluation of anticancer capacity of exotic fruits: *Nephelium Lappaceum, Melicoccus bijugatus, Manilkara zapota* via the modulation of intracellular SUMOylation processes. **Gisela M Basto-López**. Juárez Autonomous University of Tabasco

ST-6 P-Rex1, a signaling platform and effector of the Gβγ-PI3Kγ-mTORC1/2 pathway involved in cell migration. **Yarely Mabell Beltrán-Navarro.** Departments of Pharmacology and Cell Biology. CINVESTAV-IPN

ST-7 Antiproliferative effect of white and brite adipocytes co-culture with lung cancer cells A549. **Dulce María Caraveo Gutiérrez**. Universidad Autónoma de Querétaro

ST-8 Aquaporins in *Saccharomyces cerevisiae* potentially presents a differential role in the regulation of cell volume. **Chávez-Vega A**. Faculty of Natural Science. Autonomus University of Querétaro

ST-9 PKCz involvement in the HIF protein levels regulation through GSK3b during experimental renal carcinogenesis. **Patricia Curiel-Muñiz**, Departamento de Biología. Facultad de Química, UNAM

ST-10 Identification of the Guanine Nucleotide Exchange Factor VAV1 as a Novel Target of the Protein Tyrosine Phosphatase 1B. **Olga Villamar Cruz.** Unidad de Investigación en Biomedicina, Facultad de Estudios Superiores Iztacala, UNAM

ST-11 Activation of the MAP Kinase Pathway by Testosterone and DHT is Dependent on Src/EGF-R but Independent on the Intracellular Androgens Receptor in C2C12 Muscle Cells. **Dennys Paola Ferreyra-Picazo**. CINVESTAV-IPN

ST-12 Effect of Human Serum on IR/Akt/p70S6K Signaling in Breast Cancer Cells. **Laura C. Flores García**, Instituto de Investigaciones Biomédicas UNAM

ST-13 Resistin induces an epithelial to mesenchymal transition-like process in mammary epithelial cells MCF10A. **Octavio Galindo Hernández.** Departamento de Bioquímica, Facultad de Medicina, Universidad Autónoma de Baja California

ST-14 Expression of TF antigen and moesin in MCF-7 cells stimulated with LPS. **Luis Miguel García Cruz**. Instituto Tecnológico de Oaxaca-Tecnológico Nacional de México

ST-15 Caracterization of LjROPs interactome change in *Lotus japonicus* roots upon inoculation by symbiotic bacteria *Mesorhizbium loti*. **Ivette García Soto**. Centro de Ciencias Genómicas, UNAM

ST-16 Regulation of the Wnt/β-catenin pathway in hepatocellular carcinoma by an adenosine derivative. **Nuria Guerrero-Celis**. Instituto de Fisiología Celular, Departamento de Biología Celular y del Desarrollo, UNAM

ST-17 IL-2 rescues peripheral blood mononuclear cells from apoptosis induced by cervical cancer cells. **Adriana Gutiérrez-Hoya**. Laboratorio de Oncología Molecular, FES Zaragoza, UNAM

ST-18 Capsaicin Inhibit both force production of isolated skeletal muscle and physical performance of intact mice in an administration route dependent manner. **Ana Maria Guzman Ambriz**. Universidad de Colima,

ST-19 Structural and functional analyses of mammalian sperm-specific Na+/H+ Exchanger (sNHE) imply that the mammalian sNHEs are differently regulated according to species. **Sandra Hernández-Garduño.** Instituto de Biotecnología, UNAM

ST-20 Evaluation of the protein expression and localization of Retinoblastoma mutants. **Jesús Hernández-Monge**. Instituto de Física, UASLP

ST-21 Impact of *O*-GlcNAcylation over the PI3-kinase/Akt pathway in Oral Squamous Cell Cancer. **Daniela Illescas Barbosa.** Faculty of Medicine, Autonomous University Benito Juárez of Oaxaca

ST-22 The G-protein β subunit, Gpb1, regulates virulence in *Mucor circinelloides*. **Marco Iván Valle-Maldonado**. Laboratorio de Diferenciación Celular, Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo

BASIC BIOCHEMISTRY II

B-30 Deletion of the complex V-dimerizing subunit g and its effect on the mitochondrial bioenergetics in *Ustilago maydis.* **Mercedes Esparza-Perusquía**. Bioquímica, Facultad de Medicina, UNAM

B-31 Atomic structure of Ornithine Decarboxylase from *Saccharomyces cerevisiae*: Solving the mechanism of its dual activity**. Jessica Georgina Filisola Villaseñor**. CINVESTAV Zacatenco

B-32 Effect of heavy metals on the ATPase activity of the V_2 and V_1 from *Ustilago maydis*. **Giovanni García-Cruz**. Departamento de Bioquímica. Facultad de Medicina, UNAM

B-33 Non-classical hydrophobic effect in the recognition of hydrophobic ligands by bovine odorant binding protein. **Enrique García Hernández.** Instituto de Química, UNAM

B-34 Hypoxia effects on matrix metalloproteinases expression in lung cancer. **Antonio Armando García Hernández**. National Institute of Respiratory Diseases

B-35 Analysis of *in vivo* interaction of Ribonuclease II, Ribonuclease PH, and RNA Degradosome in *Escherichia coli*. **Jaime García Mena**. Genética y Biología Molecular. CINVESTAV Zacatenco

B-36 Glucose-6-phosphate dehydrogenase (G6PD) deficient; a biochemical perspective. **Saúl Gómez-Manzo.** Laboratorio de Bioquímica Genética. Instituto Nacional de Pediatría

B-37 New structural insights regarding the binding mode of the Kunitz-type protease inhibitors: canonical or non-canonical subclassification. **Yasel Guerra.** Instituto de Biotecnología, UNAM

B-38 Spectroscopic studies show evidence that curcumin derivatives inhibit TGR of *Taenia crassiceps* cysticerci. **Alberto Guevara-Flores.** Departamento de Bioquímica, Facultad de Medicina, UNAM

B-39 Function of the Cox1 carboxyl terminal-end in yeast phenotype and mitochondrial function. **Ana Paulina Gutiérrez-Alejandre.** Genética Molecular, Instituto de Fisiología Celular, UNAM

B-40 Biochemical characterization of *Rhinella marina* skin secretions and identification of enzymatic activities. **Itzel Amairani Gutiérrez Reyna.** Centro de Investigación en Biotecnología, UAEM

B-41 Biochemical characterization of HSL *BaEstB* esterase from *Bjerkandera adusta* and its natural substrates. **Daniel Hernández Fuentes.** Centro de Investigación en Biotecnología, UAEM

B-42 Purification and characterization of novel metaloproteases from *Holothuria inornata* from Chamela Bay, Jalisco, Mexico. **Alan G. Hernández-Melgar.** Departamento de Química de Biomacromoléculas. Instituto de Química, UNAM

B-43 Immunophenotype of senescent cells. **Elisa Hernández Mercado.** Laboratorio de Bioenergética y Envejecimiento Celular. Posgrado en Biología Experimental UAM-I

B-44 Quantification of gibberellic acid 3 (GA3) in liquid and powder matrix fertilizers by high performance liquid chromatography (HPLC). **Gabriel Hernández Morales**. Tecnológico Nacional de México en Celaya

B-45 Biochemical characterization of recombinant lactate dehydrogenase-1 from white shrimp *Litopenaeus vannamei.* **Magally L.E. Hernández Palomares**. Centro de Investigación en Alimentación y Desarrollo, A.C

B-46 Human Papilloma Virus-18 uses a novel type of mRNA to drive translation of oncoprotein E6 during tumor development. **Greco Hernández.** Division of Basic Research, National Institute of Cancer

B-47 Effect of male reproductive tract secreted proteins on human sperm function. **Gabriela Hernández-Silva**. Departamento de Biología de la Reproducción, Instituto Nacional de Ciencias Médicas y Nutrición. "Salvador Zubirán"

B-48 Proline Content in Commercial Wines. **Jesús Abelardo Salazar Alanis.** Departamento de Química. Facultad de Ciencias Biológicas. Universidad Autónoma de Nuevo León

B-49 Proteomics based on LC-MS/MS in tissues of patients chemoresistant to platinum and taxanes with ovarian epithelial cáncer. **Gloria Angelina Herrera Quiterio.** Faculty of Chemical-Biological Sciences, Autonomous University of Guerrero

B-50 Role of subcellular localization in the subfunctionalization of the paralogous proteins Leu4 and Leu9 in *Saccharomyces cerevisiae*. **Jaqueline Hersch González.** Instituto de Fisiología Celular, UNAM

B-51 Evaluation of the effect of heat capacity on the catalysis of a dimeric enzyme. **Ekaterina Jalomo Khayrova**. Instituto de Biotecnología, UNAM

B-52 Study of the transcriptional regulation of paralogous genes LEU4 and LEU9 in *Saccharomyces cerevisiae*. **Angélica Mariana Jara Servín.** Instituto de Fisiología Celular. UNAM

B-53 NaTrxh, a secreted thioredoxin type h from Nicotiana alata contains a rare signal peptide possibly recognized only by plant cells. **Diana Guadalupe Arzate Peña**. Departamento de Biología Comparada. Facultad de Ciencias, UNAM

B-54 Effect of Iztli peptide 1 on CELL-CYCLE arrested mammalian cells. **Maria Teresa Lara Ortiz**. Departamento de Bioquímica y Biología Estructural. Instituto de Fisiología Celular, UNAM

B-55 Effect of moderate exercise and metformin treatment on mitochondria isolated from old rats quadriceps. **Stefanie Paola López-Cervantes.** Departamento de Ciencias de la Salud. Universidad Autónoma Metropolitana, Unidad Iztapalapa

B-56 Single residue change causes loss in phospholipase and hemolytic activity on *Vibrio paraheamolyticus* thermolabile hemolysin. **Alonso Alexis López-Zavala.** Laboratorio de Investigación en Alimentos. Universidad de Sonora

B-57 Switch expression of MATs proteins in a sequential model of cirrhosis-hepatocellular carcinoma induced by DEN and the hepatoprotective effects of IFC305. **María Guadalupe Lozano-Rosas**. Instituto de Fisiología Celular, UNAM

B-58 Contribution of the Mitochondrial Intrinsic Pathway to the Apoptosis Resistance exhibited by Fibroblasts from Human Idiopathic Pulmonary Fibrosis. **E. R. Luis-García.** Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas

BIOTECHNOLOGY II

BT-36 Microbial growth phases of *Bacillus thuringiensis* characterized by online permittivity and conductivity measurements. **Escalante-Sánchez Abdi.** Departamento de Biotecnología y Bioingeniería. CINVESTAV IPN

BT-37 Structural features involved in the hemolytic activity of a pepper defensin. **Georgina Estrada Tapia.** Unidad de Bioquimica y Biologia Molecular de Plantas. Centro de Investigacion Cientifica de Yucatan

BT-38 Restructuring of the rhizospheric microbiome of *Musa acuminata* (banana) cultivated in Southeastern Mexico, upon soil inoculation with microbial consortia. **Elisa Fernández-Castillo.** CIATEJ

BT-39 Evaluation of toxic activity of reactivated *Bacillus thuringiensis var israelensis* from fermentation extracts, after more than three decades of storage and analysis of toxic persistance against *Aedes aegypti* larvae. **David Daniel Fernández Chapa**. UANL

BT-40 Carotenoid production in cultures of *Phaffia rhodozyma* under magnesium limited conditions. **Iris Isabel Flores Manzanero**. CINVESTAV IPN Zacatenco

BT-41 A novel alcoholic functional beverage made from *Agave salmiana* and probiotic mixed culture. **Alejandro García Cruz.** Departamento de Ingeniería en Biotecnología, Universidad Politécnica Metropolitana de Puebla

BT-42 *Opuntia ficus*-indica nanofibers as drug release system. **Elsy J. García-Valderrama.** Centro de Innovación en Diseño y Tecnología

BT-43 Actinomycetes from natural areas with promoting growth plant capacity. **Blanca Estela Gómez Luna.** University of Guanajuato

BT-44 Relevance of Tal2a, a putative LysM effector from *Trichoderma atroviride*, on its activity as mycoparasite and plant endosymbiont. **Sandra M. Gómez-Méndez.** Departamento de Biología, Universidad de Guanajuato

BT-45 Degradation in hypersaline conditions of aromatic polycyclic hydrocarbons and pharmaceutical compounds by the halophilic strains *Aspergillus sydowii*-like H1 and *Aspergillus destrudens* EXF-10411. **Deborah González Abradelo.** UAEM

BT-46 Design and production of CRISPR-dCas9 and dCas12a dimers for the formation of nanostructurs with DNA. **Itzel G. González Carmona.** Instituto de Química, UNAM

BT-47 Production in E. coli and IMAC purification of the C-terminal region of the *Schizosaccharomyces pombe* FXNA peptidase. **González Esparragoza Dalia.** Centro de Química. Instituto de Ciencias BUAP

BT-48 Characterization of protease activities in mid-gut of *Chrysoperla carnea* larvae. **Claudia de la Paz González García.** Universidad de Guanajuato

BT-49 Isolation of halotolerant bacteria, biosurfactant producer. **Jorge Gracida.** Facultad de Química. Universidad Autónoma de Querétaro

BT-50 Manufacture of DNA microarray for identification of transgenic organisms in maize. **Simón Guzmán León.** Unidad de Microarreglos de DNA, Instituto de Fisiología Celular, UNAM

BT-51 Caffeic Acid Production from Coumaric Acid in a Recombinant *Escherichia coli* Strain. **Georgina Hernández Chávez.** Instituto de Biotecnología, UNAM

BT-52 Cultivation of the cyanobacterium *Desertifilum* sp. for the production of biomass and C-Phycocyanin. **Ingrid Hernández Martínez.** Maestría en Ciencias Naturales e Ingeniería, Universidad Autónoma Metropolitana-Cuajimalpa

BT-53 Evaluation of ptsG and galR deletions on glucose transport and catabolic repression in a lactogenic strain of *Escherichia coli*. **Tlakaelel Akolmiztli Hernández Ríos.** Instituto de Biotecnología, UNAM

BT-54 Leucine aminopeptidase yspII from *Schizosaccharomyces pombe* involved in mitosis. Revertant of the mutant strain (4XΔape2). **Irma Herrera Camacho.** Centro de Química del Instituto de Ciencias, BUAP

BT-55 Effect of nutritional and physicochemical factors on the dimorphic transition and production of α-amylase in the yeast *Candida wangnamkhiaoensis*. **Raziel Arturo Jiménez Nava.** Escuela Nacional de Ciencias Biológicas. IPN

BT-56 Synthesis of caffeic acid using variables of cytochrome BM3. **Jorge Luis Jiménez Niebla.** Instituto de Biotecnología, UNAM

BT-57 Isolation of three new novel nor-triterpenes with anti-inflammatory activity from a population of the Mexican species *Galphimia glauca* Cav. grown on the state of Morelos. **Eleazar León-Álvarez.** Centro de Investigación en Biotecnología, UAEM

BT-58 Callus induction and phytochemical profile of *Jatropha curcas* L. **Gerardo Leyva Padrón.** Centro de Desarrollo de Productos Bióticos. IPN

BT-59 Physiological and molecular characterization of *Saccharomyces cerevisiae* strains for Tequila production. **Araceli López Andrade**. Universidad de Guanajuato

BT-60 Modulation of the activity and expression of the peroxidase ZmPrx35 from insect-resistant maize endosperms (*Zea mays* L.; P84C2R) in response to mechanical and insect damage. **Laura Margarita López-Castillo.** Tecnológico de Monterrey

BT-61 Generating a diagnostic method for differentiation of all four serotypes of dengue virus by PCR, using samples Tabasco State. **López Mondragón Daniela Alejandra.** Universidad Juárez Autónoma de Tabasco

BT-62 Efficient pyruvate production from glucose and xylose using metabolically engineered *Escherichia coli* and limited oxygen transfer conditions. **Mauricio López Portillo Masson.** Instituto de Biotecnología, UNAM

BT-63 Analysis of molecular dynamics in modified sugar transporters for bioethanol production. **Aline López-Vargas.** Autonomous University of Querétaro

BT-64 Design and construct of two modules to detect Listeria monocytogenes using *Escherichia coli* and *Bacillus subtilis* as chassis. **Celia Monserrat Luna-Castro.** Universidad de Guanajuato, Campus Irapuato-Salamanca

BT-65 Micropropagation from nodal segments of *Vaccinium corymbosum*. **María del Mar Machado Achirica.** Unidad Profesional Interdisciplinaria de Ingeniería Campus Guanajuato. IPN

BT-66 Induction of hairy roots by three strains of *Agrobacterium rhizogenes* on *Agastache Mexicana*. **Angélica Lucía Martínez Aguilar.** Centro de Investigación en Biotecnología. Laboratorio de Botánica Estructural. UAEM

BT-67 Removal of chromium VI by fungal isolated associated to lichens. **Fernando Martínez-Armenta.** Instituto Tecnológico Superior de Irapuato

BT-68 UTMBioLAB: Molecular-Biotechnological Diagnostic. **Eber Diego Martínez Correa.** Universidad Tecnológica de Morelia

BT-69 Biochemical study of the production of melanin in the mutant ΔPMA1 of *Ustilago maydis* and its potential use in bioremediation. **Martínez López Cinthya.** Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, IPN

BT-70 Heterologous production of caffeic acid in recombinant *Escherichia coli***. Luz Maria Martínez Mejía.** Instituto de Biotecnología, UNAM

REACTIVE OXYGEN SPECIES I

ROS-1 Evaluation of the energetic metabolism and redox state of senescent breast epithelial MCF-10 cell line. **Angélica Alejandra Aquino-Cruz.** UAM Iztapalapa

ROS-2 Changes in expression in the EhTRF-like proteins of *Entamoeba histolytica* during oxidative stress. **Karen Anell Becerril Puente.** Posgrado en Ciencias Genómicas, Universidad Autónoma de la Ciudad de México

ROS-3 Antioxidant enzymatic system of the antarctic yeast *Rhodotorula mucilaginosa* M94C9; a biochemical and bioinformatic – comparative study. **Jorge Brito Sánchez.** Facultad de Ciencias, UNAM

ROS-4 Combination of single doses of Cytarabine and Ferric Carboxymaltose (Fe+3) increases oxidative damage and alter redox balance in rat brain. **David Calderón Guzmán.** Medicine Experimental Division. National Institute of Pediatrics

ROS-5 Expression of peroxyrredoxin 3 in squamous intraepithelial lesions and cervical cancer. **Ruth Yareli Calvario Pino.** Facultad de Ciencias Químico Biológicas. Universidad Autónoma de Guerrero

ROS-6 *Debaryomyces hansenii* catalase T gene overexpression on an acatalasemic strain of *Saccharomyces cerevisiae*. **Román Alfonso Castillo Díaz.** Laboratorio de Biología Molecular y Genómica, Facultad de Ciencias, UNAM

ROS-7 Impact of Resveratrol on the temporal lobe of Wistar rats treated during the aging process. **Iván Cesar Arteaga**. Departamento Bioquímica-Alimentos, Facultad de Ciencias Químicas. BUAP

ROS-8 Variants in SOD2, CAT, GPX1 and GPX7 genes as markers of oxidative stress associated with obesity in children from Mexico City. **Cota-Magaña Ana Isabel.** Doctorado en Ciencias Biológicas y de la Salud, UAM Iztapalapa

ROS-9 Determination of antioxidant capacity in the organic waste of Tenebrio molitor larvae .**Dinora Guadalupe Díaz-Parra.** Chemistry Department. CUCEI, Universidad de Guadalajara

ROS-10 Protective effect of achiote extracts against different types of stress in the model of *C. elegans*. **Darío Gómez-Linton.** Ciencias de la Salud. Universidad Autónoma Metropolitana, Unidad Iztapalapa

ROS-11 Effect of hypoxia, reoxygenation, temperature and silencing on the expression of white shrimp *Litopenaeus vannamei* manganese superoxide dismutases. **Carlos Ricardo González-Ruiz.** Centro de Investigación en Alimentación y Desarrollo

ROS-12 Analysis of the activity of phenolic bean compounds in oxidative stress tolerance in Caenorhabditis elegans. **Alejandra Yitzel Guzmán Hernández**. Instituto de Investigaciones Químico Biológicas, Universidad Michoacana.

ROS-13 Resveratrol regulates oxidative markers and antioxidant enzymes during the aging process. **Daniel Juárez Serrano.** Departamento Bioquímica-Alimentos, Facultad de Ciencias Químicas. BUAP

ROS-14 Stress-induced premature senescence (SIPS) of Primary Prostate Epithelial Cells (HPEC) and Evaluation of Senescence-Associated Secretory Phenotype (SASP). **Verónica Salas-Venegas.** Posgrado en Biología Experimental, UAM Iztapalapa

GENETICS, EPIGENETICS AND GENETIC REGULATION II

GR-30 Cysteine-rich receptor-like kinase gene family identification in *Phaseolus* genome and functional characterization of *PvCRK26* and *PvCRK46* during rhizobial symbiosis. **Gabriel-Xicoténcatl García.** Ciencias Agrogenómicas, ENES Unidad León UNAM

GR-31 Sodium butyrate effect on growth and dimorphic transition of *Yarrowia lipolytica*. **Juan Manuel González Prieto.** Biotecnología Vegetal. Centro de Biotecnología Genómica. IPN

GR-32 Analysis of SNP's in the *Pun1* Gene (*Capsaicin synthase*) in some cultivars of *Capsicum annuum* of Mexico. **Alberto González Zamora.** Laboratorio de Biología Evolutiva. Facultad de Ciencias Biológicas. Universidad Juárez del Estado de Durango

GR-33 Functional diversification of paralogous α-Isopropylmalate synthases from the ancestral type yeast *Lachancea kluyveri*. **Estefany Granados Avalos.** Instituto de Fisiología Celular, UNAM

GR-34 Genetic evidence for homodimerization of the proteins encoded by essential genes RHE_PE00001 and RHE_PE00024 in *Rhizobium etli* CFN42. **Carmen Guadarrama.** Programa de Ingeniería Genómica. Centro de Ciencias Genómicas, UNAM

GR-35 *BDNF* SNP´s are Associated with Body Composition and Bone Mineral Density in Postmenopausal Women. **Guerrero-Contreras Israel.** Facultad de Salud Pública y Nutrición. Universidad Autónoma de Nuevo León

GR-36 *CgABF1* is an important regulator of adherence, chronological life span and oxidative estress response in *Candida glabrata*. **Ma. Guadalupe Gutiérrez-Escobedo.** Instituto Potosino de Investigación Científica y Tecnológica, AC

GR-37 ¿Is there a relationship between genetic variability and viability loss of the *Escontria chiotilla* and *Stenocereus pruinosus* (Cactaceae) seeds? **David Alejandro Guzmán-Hernández.** Universidad Autónoma Metropolitana Iztapalapa

GR-38 Effect of bisphenol A in steroid hormone receptors methylation patterns. **Luis Ricardo Hernández Barrientos**. Faculty of Chemistry, UNAM

GR-39 AMPK modulates the genetic and metabolic effects of biotin deficiency. **Alain Hernández-Vázquez.** Unidad de Genética de la Nutrición, Instituto de Investigaciones Biomédicas, UNAM. Instituto Nacional de Pediatría

GR-40 Search of the components of 5' to 3' and 3' to 5' pathways of mRNA decay in *Ustilago maydis.* **José Juan Jacinto Vázquez.** Posgrado en Microbiología, Instituto de Ciencias, BUAP

GR-41 A member of ANR family modulates the expression of genes regulated by PerA in enteropathogenic *Escherichia coli*. **Juan Bernardo Jaramillo-Rodríguez.** Licenciatura en Biomedicina-BUAP

GR-42 Interaction of the BER pathway with Transcriptional Factors Mfd and GreA and its consequences on *Bacillus subtilis* Adaptive Mutagenesis. **Hilda Cecilia Leyva-Sánchez.** Departamento de Biología, Universidad de Guanajuato

GR-43 UVB and UVC inhibts cellular processes related to carcinogenesis in cervical cancer cell lines. **Jesús Adrián López.** Unidad Académica de Ciencias Biológicas, Universidad Autónoma de Zacatecas

GR-44 Effect of folic acid on the regeneration of *Lumbriculus variegatus*. **López Martínez Juana María**. Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro

GR-45 Characterization of *Sporothrix schenckii* strains silenced in the gene encoding for Gp70. **López Ramírez, L.A**. División de Ciencias Naturales y Exactas. Universidad de Guanajuato

GR-46 Reconstruction of pantothenate synthesis pathway in rhizobia: where does β-alanine come from? **Mariana López Sámano.** Centro de Ciencias Genómicas, UNAM

GR-47 Mutations in the RB1 gene of Mexican childrens with retinoblastoma. **Antonio Loreto Velázquez.** Instituto de Física, Universidad Autónoma de San Luis Potosí

GR-48 Papilloma virus and its potential role in retinoblastoma tumors in Mexican population. **Jesús Adrián Lozano Sánche**z. Laboratorio de interacciones moleculares y cáncer. Instituto de Física, UASLP

GR-49 Genetic interactions among members of the RNA-directed DNA methylation pathway during reproductive development in Arabidopsis. **Judith Lua-Aldama.** Grupo de Desarrollo Reproductivo y Apomixis. LANGEBIO. CINVESTAV

GR-50 Hypermethylation of *PPP2R2B* represents a novel mechanism by which chronic inflammation perpetuates itself. **Iris K. Madera-Salcedo.** Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán

GR-51 siRNA molecules derived from the dsRNAs synthesized by sense and antisense transcription generate gene silencing in *Giardia lamblia.* **Jaime Marcial-Quino.** Laboratorio de Bioquímica Genética. Instituto Nacional de Pediatría

GR-52 Transcriptional divergence in homeologous genes *ALT1* and *ALT2* in *Saccharmomyces cerevisiae*. **Dariel Márquez**. Department of Biochemistry and Structural Biology, Instituto de Fisiología Celular, UNAM

GR-53 Two essential genes on the secondary chromosome p42e of *Rhizobium etli* CFN42 participate in cell division. **Sofía Martínez-Absalón.** Programa de Ingeniería Genómica, Centro de Ciencias Genómicas UNAM

GR-54 Effect of ferulic acid on the expression of structural and regulatory genes for the biosynthesis of mycotoxin fumonisin B1 in *Fusarium verticillioides*. **Francisco Javier Martínez Fraca**. Facultad de Química, UNAM

GR-55 A Phasin protein involved in bioplastic metabolism in *Azospirillum brasilense* Sp7. **María de los Ángeles Martínez Martínez.** Instituto de Ciencias. BUAP

GR-56 The c-di-GMP protein MucR is necessary for cyst formation but not for alginate synthesis in *Azotobacter vinelandii.* **Iliana Chantal Martínez Ortíz.** Centro de Ciencias Genómicas, UNAM

GR-57 Expression of the small RNAs CrcZ and CrcY regulate Carbon Catabolite Repression in the Nitrogen Fixing Bacterium *Azotobacter vinelandii*. **Marcela Martínez Valenzuela.** Instituto de Biotecnología, UNAM

GR-58 Role of ExoA and PolA in *Bacillus subtilis* YwqL-dependent DNA deamination-induced repair. **Jimena Meneses Plascencia.** Departamento de Biología, Universidad de Guanajuato

MEDICINE HEALTH & NUTRITION II

MH-29 Differences on vascular reactivity and RNAm expression of Renin-Angiotensin and Kinin-Kallikrein systems components on acute and chronic myocardial infarction in male and female Wistar rats. **Jazmín Flores Monroy**. FES Cuautitlan, UNAM

MH-30 Association of the polymorphism rs9939609 in the FTO gene with type 2 diabetes in the state of Guerrero. **Daicy Grisel Francisco Aguilar**. Faculty of Biological Chemistry Sciences. Autonomous University of Guerrero

MH-31 Evaluation of arginase activity in patients with Diabetes from the Yanga General Hospital in Córdoba, Veracruz, Mexico. **Moisés Franco Rodríguez**. División Académica de Ciencias Básicas. Universidad Juárez Autónoma de Tabasco

MH-32 Metatranscriptional characterization of the intestinal microbiota in health and obese with metabolic complications children. **Luigui Gallardo-Becerra**. Instituto de Biotecnología, UNAM

MH-33 Comparative effects of colombian agraz consumption on triglycerides and oxidation markers in men and women with metabolic syndrome. **Yeisson Galvis**, School of Microbiology. University of Antioquia

MH-34 Proteomic analysis of the extracellular vesicles secreted by hepatoma cell lines: potential clinical- and pathophysiological-implications. **Rosendo García Carrillo**. Centro Universitario de Investigaciones Biomédicas, Universidad de Colima

MH-35 Expression of steroid sulfatase in epithelial ovarian cancer. **Paulina García de Alba**. Departamento de Embriología. Facultad de Medicina, UNAM

MH-36 Evaluation of the anticancer effects of quercetin and its fermentation products in human colon cells treated with bisphenol A. **Nataly García Gutiérrez**. Autonomous University of Queretaro

MH-37 Delivery of Resveratrol to cardiac cells through polymeric nanovectors. **Paola García Medina**. Escuela de Medicina y Ciencias de la Salud, Tecnológico de Monterrey

MH-38 Evaluation of the anti-migratory effect of a triple therapy in breast, colon and cervical cancer-derived cell lines. **Mical Garcia-Reyes**. FES-Iztacala, UBIMED, UNAM

MH-39 Decellularized bovine bone chips as a potential biologic scaffold. **Alejandro García-Ruiz**. Departamento de Bioquímica, Facultad de Medicina. UANL

MH-40 Differential expression of proteins in cancer cervical biopsies for the search for new biomarkers. **Olga Lilia Garibay Cerdenares**. Faculty of Chemical-Biological Sciences. Autonomous University of Guerrero

MH-41 Chromium Picolinate decreases cortisol levels in *Ovis aries*. **Andrea Gómez-Zúñiga**. Maestría en Biología de la Reproducción Animal. Universidad Autónoma Metropolitana

MH-42 LPS induce phosphorylation of RACK1 in C6/36 HT cells from *Aedes albopictus*. **Hernández-Hernández FC**. Facultad de Enfermería No.2, UAGro

MH-43 Effect of cocoa intake on the induction of *in vitro* insulin polymers in obese patients. **José Arnold González Garrido**. Universidad Juárez Autónoma de Tabasco, División Académica de Ciencias Básicas

MH-44 Restart of the estrous cycle by the estrogenic activity of the mesquite pod extract in ovariectomized rats. **Leticia González-Nuñez**. Universidad Autónoma Metropolitana, Unidad Iztapalapa

MH-45 Expression of LAMC1 and ITGβ1 in cervical-cancer-derived cell lines. **Diana Laura Gonzalez-Tolentino**. Facultad de Ciencias Químico Biólogo Parasitólogo, Universidad Autónoma de Guerrero

MH-46 Respiratory Tract Microbiota in Patients with Acute Respiratory Infections. **Guerrero Corona Diego**, Instituto Nacional de Medicina Genómica

MH-47 Effect of high fat diet and pirfenidone on gene expression involved in a mouse model of cardiomyopathy. **Jorge Gutiérrez-Cuevas**. Instituto de Biología Molecular en Medicina y Terapia Génica, CUCS, Universidad de Guadalajara

MH-48 Pharmacological Evaluation of Aqueous Extracts Of Cancerina (*Semialarium mexicanum*) as a Antiteratogenic And Cytotoxic Therapy. **Mario Javier Gutiérrez Fernández**. Universidad Tecnológica de Morelia

MH-49 Soluble Toll like receptor 2 is augmented in saliva of patients with squamous cell carcinoma. **Rebeca Guzmán Medrano**. Universidad Autónoma de Chihuahua

MH-50 Beneficial effects of exercise and metformin on the body composition of aged female Wistar rats. **David Hernández Álvarez**. Departamento de Ciencias de la Salud, Universidad Autónoma Metropolitana Iztapalapa

MH-51 Expression of BiP, IRE1, ATF6 and PERK (Unfolded Protein Response mediators) in mechanical and alkali corneal lesion in rat. **Erandeni Hernández Esparza**. Centro de Ciencias de la Salud, Universidad Autónoma de Aguascalientes

MH-52 Chemoprotective effect of lactoferrin in Hepatocellular Carcinoma. **Hury Viridiana Hernández Galdámez**. Departamento de Biología Celular. CINVESTAV. IPN

MH-53 Transcription level of socs4 and socs6 genes in peripheral blood mononuclear cells of patients with Multiple Sclerosis by real time RT-PCR. **Adriana Hernández Reyes**. Decanato de Cs. Biológicas, Universidad Popular Autónoma de Puebla

MH-54 Effect of pyrophosphate thimine on the nerve conduction speed in patients with diabetic polyneuropathy. **Itzelly Ibarra-Valdovinos**. Facultad de Medicina, Departamento de Investigación Biomédica, Universidad Autónoma de Querétaro

MH-55 Elevated arginase activity levels in patients with central obesity. **Diana Cristel Jeronimo Contreras**. The Faculty of Basic Sciences. Juarez Autonomous University of Tabasco

MH-56 Effect of the acute consumption of polyphenol and a program of physical activity in the treatment of the osteoarthritis in older adults. **Everardo Antonio Jiménez Respardo**. Escuela Superior de Medicina. IPN

MICROBIOLOGY & VIROLOGY II

MV-25 Management of patients infected with HIV in the State of Tabasco. **Luis Manuel Contreras Cabrera.** Universidad Juárez Autónoma de Tabasco

MV-26 Importance of host cholesterol in the DENV, ZIKV and YFV infection. **Carlos Daniel Cordero-Rivera.** Departamento de Infectómica y Patogénesis Molecular, CINVESTAV-IPN

MV-27 Expression of miR-142-3p and infection by *Helicobacter pylori* in patients with chronic gastritis and gastric cancer. **Ana B. Cruz Hernández**. Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Guerrero

MV-28 Comparative analysis of biofilm production by *Sporothrix schenckii* conidia and yeast-like cells. **Marilyn Gwendolyne De los Santos-Rojas**. Departamento de Biología, División de Ciencias Naturales y Exactas, Universidad de Guanajuato

MV-29 Vaccine efficacy of BCG-Phipps in bovine tuberculosis by monitoring response to ESAT-6 and CFP-10 antigens in IFN-γ release assay. **Fernando Díaz Otero.** CENID-Microbiología Animal INIFAP

MV-30 Evaluation of VP4 expression in MA-104 cell line infected with rotavirus. **Tania Guadalupe Heredia Torres.** Facultad de Ciencias Biológicas, UANL

MV-31 Participation of the pvd gene cluster in the mechanism of Quorum sensing and virulence of *Pseudomonas aeruginosa* PAO1. **Sharel Pamela Díaz Pérez**. Instituto de Investigaciones Químico Biológicas, Universidad Michoacana.

MV-32 Molecular characterization of the rOrf1 protein of enteropathogenic *Escherichia coli*. **Norma Espinosa Sánchez**. Instituto de Fisiología Celular, UNAM

MV-33 Silencing SOCS1 and SOCS3 in T Helper cells resulted in augmented Interleukin 10 production during Dengue virus infection**. Lilian Flores-Mendoza**. CIBIOR-IMSS. Ciencias Químico Biológicas, Universidad de Sonora, Unidad Regional Sur

MV-34 First report of the *Aedes aegypti* Flavivirus in field population of *Aedes aegypti* in Mexico, a mosquito-specific virus. **Suemy Flores-Ruiz.** Laboratorio de Arbovirología, Centro de Investigaciones Regionales "Dr. Hideyo Noguchi"

MV-35 Antiviral effect of a Dializable Leukocytes Extract in MDCK cells infected with influenza AH1N1 pandemic 2009. **García Martínez Daniel.** ENMyH-IPN

MV-36 Importance of co-infection of influenza virus-bactrias with host damage. **Alexis Eduardo García García**. Facultad de Medicina, UNAM

MV-37 Similar mutations in LiaR are present in lipopeptide susceptible and resistant *Enterococcus faecium* isolates. **María Carlota García-Gutiérrez.** Facultad de Medicina, Universidad Autónoma de Querétaro

MV-38 Biochemical and phylogenetic study of a flagellar lytic transglycosylase SltF from *Rhodobacter sphaeroides*. **Mariela García-Ramos.** Instituto de Fisiología Celular, Universidad Nacional Autónoma de México

MV-39 Role of PqsE in the production of pyocyanin and elastase in *Pseudomonas aeruginosa* strain ATCC 9027, which belongs to the outlier PA7 clade. **Selene García-Reyes.** Instituto de investigaciones Biomédicas, UNAM

MV-40 Identification and screening of *Trichoderma strains* natives from Querétaro with fungicide activity for biocontrol of phytopathogenic fungi. **Víctor Javier García Sánchez**. Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro

MV-41 Analysis of the co-participation between the Epstein Barr virus and the bacterium *Helicobacter pylori* in a gastric cancer model. **Ana Karen Gaytán-Jiménez**. Hospital Infantil de México Federico Gómez / UNAM

MV-42 Pasteurella multocida can use human lactoferrin as iron source by binding it to the aldehyde-alcohol-dehydrogenase. **Omar Gómez-Arenas.** FES Iztacala, UNAM

MV-43 Microbiological and biochemical study of the Killer toxin (K1) of *Saccharomyces cerevisiae* in acidic environments. **Jaqueline González-Barragán.** Basic and Applied Microbiology. Unit. Autonomus University of Querétaro

MV-44 Functional diversity of ant escamolera gut microbiota unveils key adaptations for its survival in a semiarid ecosystem. **Jorge Gonzalez-Escobar**. Department of Molecular Biology, Instituto Potosino de Investigación Científica y Tecnológica A.C.

MV-45 Norovirus and rotavirus gastroenteritis severity in children. **Guadalupe González-Ochoa**. Universidad de Sonora, División de Ciencias e Ingeniería, Departamento de Ciencias Químico Biológicas y Agropecuarias

MV-46 Biosynthesis and functions of zwitterionic membrane lipids and their hydroxylated derivatives in *Burkholderia cenocepacia* J2315. **Napoleón González-Silva**. Centro Universitario de los Altos, Universidad de Guadalajara

MV-47 Oxidative stress actives the persistence of *M. tuberculosis* in the human macrophage through WhiB3. **Silvia Guzmán-Beltrán.** Instituto Nacional de Enfermedades Respiratorias Ismael Cosio Villegas

MV-48 Proteomic analysis of *Escherichia Coli* Detergent-Resistant Membranes (DRM): An insight of membrane raft content. **José Enrique Guzman-Flores.** Instituto de Fisiología Celular. UNAM

MV-49 Analysis of biofilm growth condition by microscopy and production of indole-3-acetic acid production by *Azospirillum brasilense*. **Acatitla- Jácome Iris**. Centro de Investigaciones en Ciencias Microbiológicas, BUAP

NEUROSCIENCES AND NEUROBIOLOGY I

NN-1 Autophagy could cooperate with matrix metalloproteinases to the mouse neural tube closure. **Pilar Sarah Acevo-Rodríguez.** Departamento de Neurodesarrollo y Fisiología, División de Neurociencias, Instituto de Fisiología Celular, UNAM

NN-2 Association of miR-21 and let-7 with migration and invasion of medulloblastoma cells**. Lourdes Álvarez-Arellano.** Laboratorio de Investigación en Neurociencias, Hospital Infantil de México Federico Gómez

NN-3 Implementation of *Ex Vivo* electroretinogram (ERG) the technique in isolated mouse retinas. **Margarita Marlene Araujo Meza**. Instituto de Neurobiología, UNAM Campus Juriquilla

NN-4 Molecular and electrophysiological characterization of glutamate-gated chloride channels subunits from *Procambarus clarkii* crayfish's eyestalk. **Juan Manuel Arias Montaño.** Facultad de Estudios Superiores Iztacala, UNAM

NN-5 Expression and phosphorylation of PEBP1 in early focal brain ischemia on rat hippocampus. **Jorge Daniel Corzo Toledo.** Departamento Infectómica y Patogénesis Molecular, Cinvestav-IPN

NN-6 Effect of polypyrrole/iodine on GAP-43, GFAP and AKT expression in a model of chronic spinal cord injury in rats. **Angélica B. Coyoy Salgado**. CONACyT- IMSS

NN-7 Histological characterization involved in pathophysiology of the spinal cord injury in diabetic rats. **Adriana Domínguez-Vázquez.** Depto. de Ciencias Naturales e Ingeniería, UAM-Cuajimalpa

NN-8 P2X3 receptors participate in neuronal hyperexcitability during acute colitis. **Ismael Eduardo Gallegos Vieyra**. Universidad Autónoma de Aguascalientes

NN-9 Participation of $GABA_A$ receptors in the antidepressant-like effect of *Justicia spicigera* Schltdl in female rats in the metestrus-diestrus phases of the ovarian cycle in Wistar rats. **Rosa Isela García-Ríos.** Centro Universitario de los Lagos. Universidad de Guadalajara

NN-10 Role of Wnt signaling pathway on hippocampal reorganization after a synaptic loss. **Lizbeth E. García-Velázquez**. Department of Genomic Medicine and Environmental Toxicology, IIB, UNAM

NN-11 Pharmacological characterization of the heterologous expression of P2X1 and P2X1del human receptors. **Karen Sarahí Gómez Coronado**. Instituto Potosino de Investigación Científica y Tecnológica, A. C.

NN-12 Loss of the autophagic flux in the brain of old rats favors the establishment of cellular senescence. **Elisa Gorostieta-Salas.** Institute of Cellular Physiology, National Autonomous University of Mexico

NN-13 Differences in the plasma concentration of lipids and β-amyloid and tau protein between Mexican patients with Alzheimer's disease and healthy subjects. **Christian Guerra-Araiza.** Unidad de investigación Médica en Farmacología, CMN S XXI, IMSS

NN-14 Integrating genomic and metabolomic data to elucidate the biochemical mechanisms related to schizophrenia. **Nora Gutiérrez-Nájera.** Instituto Nacional de Medicina Genómica

NN-15 Study of neural differentiation *in vitro* using immortalized multipotent otic progenitor cells. **Edson Daniel Hernández Velázquez**. University of Guanajuato/Optics Research Center "CIO"

NN-16 Evaluation of the anti-inflammatory and antioxidant activities of scammonin I isolated from Ipomoea tyrianthina root. **Johanna Stefan Jaimes Acuña**. Centro de Investigación en Biotecnología (CEIB), UAEM

NN-17 Regulation of TRPV1 channel by endocannabinoids. **Rebeca Juárez-Contreras.** Departamento de Neurociencia Cognitiva, Instituto de Fisiología Celular, UNAM

NN-18 Characterization of corticospinal neurons from the primary motor cortex in Alzheimer's model. **Martín Macías**. Institute of Neurobiology, National Autonomous University of México

NN-19 Prolactin protection against oxidative and hypoxic stress in hippocampal neurons. **Fernando Macías Prado.** Department of Celular and Molecular Neurobiology. Neurobiology Institute, UNAM

NN-20 *Malva parviflora* extract regulates the phagocytic capacity of microglial cells via a PPARγ-mediated mechanism in an Alzheimer´s disease model**. Elisa Medrano-Jiménez.** Instituto de Biotecnología, UNAM

NN-21 Possible synaptic communication between neurons of Parafascicular and Central lateral intralaminar thalamic nuclei. **Beatríz Sarahí Méndez Rodríguez**. División de Neurociencias, Instituto de Fisiología Celular, UNAM

TOXICOLOGY I

T-1 Long term alterations produced on mice prenatally exposed to electromagnetic fields are prevented by trans-resveratrol. **Faviola Alcántara-González**. Department of Physiology, Lab. Development Pharmacology, Escuela Nacional de Ciencias Biológicas, IPN

T-2 Effect of $CdCl_2$ on the expression of DNMTs in the HepG2 cell line at subtoxic concentrations. **Alcocer Lorenzo SI**. Laboratory of Cytopathology and Histochemistry, Autonomous University of Guerrero

T-3 New interactions of PPAR gamma with glitazones, 15d-PGJ2 and TZD ring obtained from MD simulations. **Samuel Álvarez-Almazán**. Escuela Superior de Medicina. IPN

T-4 Antivenom Archeology. **Irving Archundia**. Instituto de Biotecnología, UNAM

T-5 Search and evaluation of new molecular structures with potential giardicidal activity. **Juan Carlos Auriostigue Bautista**. Laboratorio de Bioquímica-Genética, Instituto Nacional de Pediatría

T-6 Cloning, expression and purification of a metalloprotease from the venom of a pit viper endemic of Argentina. **Damaris Iyary Bolaños Guadarrama.** Instituto de Biotecnología, UNAM

T-7 Hydroxyurea interferes with the DNA damage response in Fanconi anemia cells. **José Benjamín Bustamante Gómez**. Laboratorio de Citogenética, INP

T-8 Characterization of proteins with toxic activity from venoms of *Oculicosa supermirabilis*, *Heriaeus melloteei* and *Dolomedes gertschi*. **Samuel Cardoso Arenas**. Instituto de Biotecnología, UNAM

T-9 Overexpression of the toxin MCTx-1 from the fire coral Millepora dichotoma. **Miguel Cuevas Cruz**. Instituto de Química, UNAM

T-10 Effect of phthalates (DBP and BBP) on *in vitro* expansion of human hematopoietic cells. **Víctor Balderas Hernández**. Instituto Potosino de Investigacion Cientifica y Tecnologica, A.C

T-11 Metformin, Sodium Oxamate and Doxorubicin in combination induce intrinsic apoptosis in human cervical cancer cells. **Izamary Delgado-Waldo**. Instituto Nacional de Cancerología.

T-12 Effects of atrazine on the human trophoblast. **Domínguez López Pablo E**. UIMMR Hospital de Ginecología y Obstetricia No. 4 “Dr. Luis Castelazo Ayala” IMSS

T-13 Effects of extract of *Lepidium virginicum* on DNBS-induced colitis in rats. **María Fernanda Durán Vázquez**. Centro de Ciencias Básica, Departamento de Fisiología y Farmacología Universidad Autónoma de Aguascalientes

T-14 Determinación de la actividad de una fracción menor a 1 kDa del veneno de *Palythoa caribaeorum* (Duchassaing & Michelotti, 1860) en el canal iónico Kv10.1. **Noel Fabian-Hernández**. Instituto de Química, UNAM

T-15 Indomethacin: As posible selective inhibitor of phospholipase A2. **Leticia Guadalupe Fragoso Morales**. Instituto Politécnico Nacional.

T-16 Functional expression of non-CB1 and non-CB2 cannabinoid receptor in leukemia cell lines. **Pamela Gaitán González**. Facultad de Ciencias Químicas, Universidad de Colima

T-17 Extracts of Capsicum *annum* L modulate the metabolic activity of MCF-7 and MCF-12 cell lines. **Roberto Jorge García Mendoza**. Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro.

T-18 Prevalence of polymorphisms in the human UDP-Glucuronosyl transferase UGT1A6 552A>C (Arg184Ser) in mexican mestizo population of the state of Puebla. **Wendy Argelia García Suastegui**. Departamento de Biología y Toxicología de la Reproducción, Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla

T-19 Acute intoxication with sodium nitrate produces hematological and biochemical alterations and expression of hypoxia response genes. **Rebeca Pérez Morales**. Facultad de Ciencias Químicas. Universidad Juárez del Estado de Durango

SYSTEMS BIOLOGY & BIOINFORMATICS II

SB-18 Identification of a group of microRNA's involved in the progression of breast cancer. **A.D.- Martínez Gutiérrez**. Instituto Nacional de Cancerología

SB-19 Comparative genomics analysis of seven Mexican strains of Anaplasma marginale, the first Anaplasma genomes reported in Mexico. **Fernando Martínez Ocampo**. Centro Nacional de Investigación Disciplinaria en Parasitología Veterinaria. INIFAP

SB-20 MicroRNA regulation of homoeolog genes in polyploid hybrid species. **Javier Israel Montalvo-Arredondo**. Unidad de Genómica Avanzada. LANGEBIO. CINVESTAV

SB-21 Promiscuity of Histone acetyltransferases in *Ustilago maydis*. **Jazmin Eliana Murcia Garzón.** Escuela Nacional de Ciencias Biológicas, IPN

SB-22 Secret-AAR: A Web Server to Assess the Antigenic Density of Proteins and for the Analysis of *M. tuberculosis* and *T. solium* Secretomes. **Adrián Ochoa-Leyva.** Departamento de Microbiología Molecular, Instituto de Biotecnología, UNAM

SB-23 Identification of the immunodominant peptides of the PE_PGRS33 protein of *Mycobacterium tuberculosis*. **David Ortega-Tirado.** Ciencias Químico Biológicas, Universidad de Sonora

SB-24 Functional Genomics of Germinating Sclerotia of *Sclerotium cepivorum* Berk. **John Dogulas Palleti.** Departamento de Biología. Universidad de Guanajuato

SB-25 Transcriptomic analysis during the interaction of *Clavibacter michiganensis* subsp. michiganensis with two tomato species. **Leonardo Isaac Pereyra-Bistraín**. División de Biología Molecular, IPICYT

SB-26 Halophile adaptations and stress in the lignocellulolytic fungus *Aspergillus sydowii*. **Yordanis Pérez-Llano.** Centro de Investigación en Biotecnología – UAEM

SB-27 Identification of co-expressed long non-coding RNA structural domains in similar human tissues. **Luis Jordan Perez-Medina**. Unidad de Genómica Avanzada. LANGEBIO. CINVESTAV

SB-28 Extremophile fungi: an alternative for the bioremediation of wastewater from municipal slaughterhouses in Morelos. **Verónica Ramírez Lona**. UAEM

SB-29 Aldehyde dehydrogenase diversity in bacteria of *Pseudomonas* genus. **Héctor Riveros-Rosas.** Departamento de Bioquímica, Facultad de Medicina. UNAM

SB-30 Novel lncRNAs are induced as part of the neighbor proximity response in *A. thaliana*. **Irving J. García López.** Unidad de Genómica Avanzada. LANGEBIO. CINVESTAV

SB-31 Molecular and structural modeling of HPV-16 E6 oncoprotein and variants in the interaction with P53 as target protein. **Hugo Alberto Rodríguez Ruiz.** Faculty of Chemical-Biological Sciences. Autonomous University of Guerrero

SB-32 Genomic analyses on phytopathogenic nematodes. **Luis Ángel Xoca-Orozco.** Laboratorio en Ciencias Agrogenómicas. ENES Unidad León. UNAM

SB-33 Diversity of metabolic profiles in BP8, an enriched microbial community capable of growing in a polyether polyurethane-acrylic varnish, unveiled by metagenomic analysis. **Ayixón Sánchez Reyes.** Departamento de Bioquímica. Facultad de Química, UNAM

SB-34 Prediction of the 3D structure of Aedes aegypti acetylcholinesterase. **Erika Amparo Torres Reyes**. Grupo de Investigaciones Ambientales para el Desarrollo Sostenible. Universidad Santo Tomás

BASIC BIOCHEMISTRY III

B-59 CETPI Derived Peptide Improves Acute Phase Response in a Septic Shock *in vivo* Model. **Ismael Luna Reyes.** Instituto de Fisiología Celular, UNAM

B-60 Search of small organic molecules that modulate the function of the GTPase EFL1. **Miryam Samantha Maldonado López**. Instituto de Química, UNAM

B-61 Application of a biosensor and computational tools for studies of molecular interaction of some drugs related to calmodulin. **Deyamira Matuz-Mares**. Department of Biochemistry, Faculty of Medicine, UNAM

B-62 *SLC16A11:* A transporter associated with the risk of Type 2 Diabetes in Mexicans. **Andrea Celeste Medina García**. Unidad de Biología Molecular y Medicina Genómica del IIB-UNAM/INCMNSZ

B-63 Thermodynamic and kinetic characterization of the unfolding of the B domain of the Lysine-arginine-ornithine binding protein (LAO). Comparison with the behavior of the A domain and the wild type protein. **Eva Isela Mejía Juárez**. Facultad de Medicina, UNAM

B-64 Buthionine sulfoximine is a multi-target inhibitor of trypanothione synthesis in *Trypanosoma cruzi*. **Marlen Mejia Tlachi**. Departamento de Bioquímica, Instituto Nacional de Cardiología Ignacio Chávez

B-65 Structure-based identification of a potential non-catalytic binding site for rational drug design in the fructose-1,6-biphosphate aldolase from *Giardia lamblia*. **Sara Teresa Méndez**. Laboratorio de Bioquímica-Genética. Instituto Nacional de Pediatría

B-66 Study of the putative nuclear localization signals (NLS) of the cationic channel TRPV4. **Susana Méndez-Gómez**. Departamento de Fisiología, Biofísica y Neurociencias. CINVESTAV-Zacatenco

B-67 Identification of microRNAs related to obesity in Mexican children. **Carolina Miranda-Brito.** Unidad de Investigación Médica en Bioquímica, CMN SXXI

B-68 Silencing of HIF-1 decrease the viral load and restore the antioxidant response in WSSV-infected white shrimp. **Melissa María Miranda-Cruz.** Universidad de Sonora,

B-69 Antifungal and antioxidant activity of hydrolysates from melón seed storage proteins (*Cucumis melo* L.). **Francisco Xavier Mojica Rodriguez***.* Tecnológico Nacional de México. Instituto Tecnológico de Roque

B-70 Transcriptome Sequencing by RNAseq and Differential Expression analysis to identify genes involved in Fatty Acids and Alkamides in the Mexican multifunctional plant *Heliopsis longipes.* **Jorge Molina-Torres.** CINVESTAV. Irapuato

B-71 Phenolic compounds accumulation during postharvest life in the peel of six mango cultivars. **Juan Luis Monribot-Villanueva.** Red de Estudios Moleculares Avanzados. Instituto de Ecología A. C

B-72 Molecular cloning, heterologous expression and structural modeling of Glucose-6-phosphate dehydrogenase from the protozoan *Giardia lamblia*. **Laura Eloísa Morales-Luna.** Laboratorio de Bioquímica Genética. Instituto Nacional de Pediatría

B-73 Identification of chemical compounds that bind to human Gpn1 GTPase in computational molecular docking experiments. **Julio A. Muñiz Luna**. Instituto de Física, UASLP

B-74 Common misconceptions in the usage of some evolutionary concepts in protein biochemistry. **Ricardo Muñiz Trejo**. Laboratorio de Fisicoquímica e Ingeniería de Proteínas, Departamento de Bioquímica, Facultad de Medicina, UNAM

B-75 Analysis of the impact of potassium levels on porcine kidney BADH coenzyme binding site. **César Muñoz-Bacasehua**. Centro de Investigación en Alimentación y Desarrollo AC

B-76 Function of the C-terminal domain of large-subunit catalases. **Teresa Nava Ramírez.** Biología Celular y del Desarrollo, Instituto de Fisiología Celular, UNAM

B-77 *Escherichia coli* regulates the expression of miR-146 and -451 in chorioamniotic membranes in an infection model. **Mercedes Olvera-Valencia.** Inmunobioquímica. Instituto Nacional de Perinatología Isidro Espinosa de los Reyes

B-78 Hypoxia inducible factor -1 regulates key glycolytic genes in WSSV-infected white shrimp. **Diana Lourdes Padilla-Bórquez.** Ciencias Químico-Biológicas y Agropecuarias. Universidad de Sonora

B-79 Sphingolipid biosynthesis and function in bacteria. **Jonathan Padilla-Gómez.** Centro de Ciencias Genómicas, UNAM

B-80 Structural comparison of human and murine G0S2 proteins. **Edgar Daniel Páez-Pérez.** Laboratorio de Biología Estructural, Instituto Potosino de Investigación Científica y Tecnológica

B-81 Progesterone modulates the secretion of heat shock protein (Hsp)-60 in choriodecidua tissue after stimulation with *Escherichia coli* in a model of infection. **Janelly Estefanía Palacios Luna**. ⁻Department of Inmunobioquímica Instituto Nacional de Perinatología

B-82 Mitochondrial function and dynamics in MH-S alveolar macrophages treated with P27 y PE_PGRS33 recombinant proteins of *Mycobacterium tuberculosis*. **Iris Selene Paredes González.** Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán

B-83 Exploring chemical space to find potential inhibitors of protein tyrosine phosphatase 1B. **Luis Enrique Pérez del Castillo**. Facultad de Medicina y Nutrición. Universidad Juárez del Estado de Durango

B-84 Biochemical Characterization of Two Single Mutant (A+ and Nefza) that Give Rise to a polymorphic Glucose-6-phosphate dehydrogenase A- Double Mutant. **Edson Jiovany Ramírez Nava.** Laboratorio de Bioquímica Genética. Instituto Nacional de Pediatría

B-85 Functional Studies of SRD5A2 Gene Mutations, Identified in Individuals with Steroid 5α-Reductase Type-2 Deficiency/46,XY Disorder of Sexual Development. **Luis Ramos Tavera**. Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán

B-86 Role of the aryl hydrocarbon receptor (AhR) on the oncoprotein E7-HPV16 degradation mechanism by the proteasoma 26S pathway. **Octavio Daniel Reyes Hernández.** Departamento de Bioquímica. Escuela Nacional de Ciencias Biológicas, IPN

B-87 Plasma homocysteine levels in BALB / c mice with L5178Y lymphoma. **Fabiola Rodríguez Arévalo.** Centro Universitario de Ciencias Exactas e Ingenierías, Universidad de Guadalajara

BIOTECHNOLOGY III

BT-71 Expression and purification of recombinant glucose oxidase in *Pichia pastoris* and evaluation of cytotoxic and anticancer activity in vitro. **Evelyn Martínez Mora.** Facultad de Ciencias Químicas. Universidad Autónoma de Nuevo León

BT-72 Effect of the SNF1 deletion in the glycolytic pathway of *Saccharomyces cerevisiae* grown at 1% glucose. **Cecilia Martinez-Ortiz.** Instituto Tecnológico Superior de Ciudad Hidalgo

BT-73 Isolation and *in vitro* evaluation of peptides for their potential use as immunogens against *Anaplasma marginale*. **Julián Martínez Salgado.** Maestría en Biotecnología. CEIB - UAEM

BT-74 Degradation of diesel and waste oil by *Pseudomonas spp.* isolated from contaminated soil. **José Antonio Martínez Villalba**. Departamento de Ingeniería Ambiental. Universidad Iberoamericana Torreón

BT-75 Study on the saccharification of the lignocellulosic residue from the processing of jackfruit (*artocarpus heterophyllus*). **Medina-Morales José Uriel.** Programa de Maestría en Ciencias Biológico Agropecuarias. Universidad Autónoma de Nayarit

BT-76 Evaluation of the phenotypic and molecular response of the interaction *Capsicum annum* L- peroxyacetic acid – geminiviruses. **Mejía-Teniente Laura.** CA. de Biotecnología, Sustentabilidad e Ingeniería. Universidad de Guanajuato

BT-77 Stimulation of TOR signaling pathway by the rhizobacteria *Azospirillum brasilense* in Arabidopsis. **Manuel Méndez Gómez.** Instituto de Investigaciones Químico Biológicas. UMSNH

BT-78 *Pv*KNOLLE: a molecular marker for the *in vivo* visualization of the cytokinesis in *Phaseolus vulgaris* roots. **Elizabeth Monroy Morales.** Departamento de Biología Molecular de Plantas, Instituto de Biotecnología, UNAM

BT-79 Analysis of diclofenac biodegradation in a continuous bioreactor by free cells of ascomycetes fungus. **Dario Rafael Olicón-Hernández.** Institute for Water Research, University of Granada, Spain

BT-80 Phenylpropanoids associated with the induction of embryogenic response in cultures of avocado (*Persea americana* Mill). **Carol A. Olivares-Garcia.** Unidad de Investigación y Desarrollo de Alimentos. Instituto de Ecología A.C

BT-81 Understanding the thermal inactivation kinetics of the NADH Oxidase from *Giardia lamblia*. **Jesús Oria Hernández***.* Laboratorio de Bioquímica-Genética. Instituto Nacional de Pediatría

BT-82 Characterization of a 3d Construction with movilized sheep bone marrow cells differentiated to bone and cartilage. **Carmina Ortega Sanchez.** Instituto Nacional de Rehabilitacion “Luis Guillermo Ibarra Ibarra

BT-83 *Azotobacter vinelandii* mutant in relA/spoT homologous promotes early degradation of PHB. **Cristian Camilo Ortiz Vasco.** Instituto de Biotecnología-UNAM

BT-84 Untargeted and targeted metabolomics analysis reveals a possible role of flavin monooxygenase YUCCA4 in the production of glucosinolates and plant development in Arabidopsis. **Randy Ortiz-Castro.** Instituto de Ecología, A.C

BT-85 Sea cucumbers as a potential marine collagen source: a high performance method. **Jorge A. Osorio-Kuan.** Departamento de Química de Biomacromoléculas, Instituto de Química, UNAM

BT-86 The use of a radio-sterilized fascia lata as a scaffold of the mesenchymal stem cells derived from the adult Hoffa´s fat package. **Ma. Dolores Ozuna Arroyo**. Instituto Nacional de Rehabilitación

BT-87 Functional characterization of two patatin-related phospholipases A (pPLA) of *Phaseolus vulgaris* during nodulation. **Ronal Pacheco.** Departamento de Biología Molecular de Plantas, Instituto de Biotecnología. UNAM

BT-88 Biochemical Characterization of Wild Yeasts Producing Ethanol. **Román Bruno Paz García**. Facultad de Ciencias Biológicas, Benemérita Universidad Autónoma de Puebla

BT-89 *In vitro* production of Huperzine A by cell suspension cultures of the plant species *Huperzia orizabae*. **Rocio del Carmen Pérez Aguilar.** Centro de Investigación en Biotecnología. UAEM

BT-90 Electrochemical analysis of the redox metabolites produced by *Pseudomonas aeruginosa* NEJ01R. **Pérez-Garcia, Jesús Alberto. C**entro de Investigación y Desarrollo Tecnológico en Electroquímica, S.C.

BT-91 Biodegradation of bisphenol a by *Pleurotus ostreatus* grown in submerged fermentation and effect on laccase enzymes activity. **Georgina Pérez-Montiel.** Autonomous University of Tlaxcala

BT-92 Effect of the extracts of the plants Bouvardia ternifolia and *Solanum nigrescens* on the growth of *Escherichia coli*. **Georgina Pérez-Montiel.** Laboratorio de Biología Experimental. Facultad de Agrobiología, Universidad Autónoma de Tlaxcala

BT-93 Entomochemicals present in different phenological states of the Central American locust (*Schistocerca piceifrons piceifrons*, Walker). **Rogelio Pérez-Ramírez.** Instituto Tecnológico de Ciudad Victoria-División de Estudios de Posgrado e Investigación

BT-94 Partial purification of an intracellular β-glucosidase from *Clavispora lusitaniae*. **Odilia Pérez-Avalos.** Department of Biotechnology and Bioengineering. CINVESTAV-IPN

BT-95 Physiological role of cytokinins in *Trichoderma atroviride*. **María Daniela Porras Troncoso.** Laboratorio Nacional de Genómica para la Biodiversidad. CINVESTAV IPN

BT-96 Shrimp waste used for biotechnological purposes. **Laura Itzel Quintas Granados.** Universidad Mexiquense del Bicentenario Unidad de Estudios Superiores Tultitlán

BT-97 Regulated plasmid construction to express a bioactive lectin of *Phaseolus acutifolius* in *Trichoderma reesei*. **Ramírez-Baltazar S.** Unidad de Microbiología Básica Aplicada, Facultad de Ciencias Naturales. Universidad Autónoma de Querétero

BT-98 *In silico* analysis of a metagenomic obtained from marine sponges: A lignocellulolytic prospection. **Daniela D. Ramírez-Escamilla.** Universidad Autónoma del Estado de Morelos

BT-99 Characterization of cell wall resident proteins NCW-3, ACW-1 and CCG-6 as possible anchors for protein display in *Neurospora crassa*. **Ana Sofía Ramírez-Pelayo.** Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco

BT-100 Fungal-biosynthesized silver nanoparticles (AgNPs) and its use to prevent biofilm formation of Uropathogenic *Escherichia coli* (UPEC). **Rosa María Ramírez Santoyo.** Unidad Académica de Ciencias Biológicas, Universidad Autónoma de Zacatecas

BT-101 Efficiency comparison of polyclonal antibodies against MDM2 made in rabbit and chicken. **Yolanda Rebolloso-Gómez.** Lab. de Interacciones Biomoleculares y Cáncer. Instituto de Física, UASLP

BT-102 Heterologous expression of an antimicrobial peptide generated by bioinformatic analysis of anurans sequences. **Diana Laura Resendiz Torres.** Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos

BT-103 *In vitro* probiotic assessment of Pantoea dispersa isolated from aguamiel and pulque. **Allison Vianey Valle Bravo.** Universidad Politécnica del Estado de Morelos

BT-104 **RETIRADO POR EL TUTOR**

BT-105 Desing of a system of temporary immersion of *in vitro* cultures of plants by gravity at laboratory level. **José Ramón Rodríguez Vázquez.** Unidad Profesional Interdisciplinaria de Ingeniería. Campus Guanajuato. IPN

BT-106 Coupling planar chromatography directly to ambient ionization Mass Spectrometry (AIMS) for Phytochemical Profile Analysis. **Nancy Shyrley Garcia-Rojas.** CINVESTAV Unidad Irapuato

BT-107 Cloning and expression of the *fur* $_{GDI\_1248}$ gene of *Gluconacetobacter diazotrophicus* Pal 5. **Roldan-León B. E.** Laboratory of Microbial Physiology of the Interaction Microorganism-Host. CICM-ICUAP. BUAP

BT-108 Development of a new viral vector system for overexpression of recombinant proteins of pharmaceutical interest in *Chlamydomonas reinhardtii*. **Alejandro Romo-Avalos.** Instituto Potosino de Investigación Científica y Tecnológica, A.C

GENETICS, EPIGENETICS AND GENETIC REGULATION III

GR-59 Differential expression of components of the hepatic glutamatergic system in cirrhosis and hepatocarcinoma. **Isabel Méndez.** Instituto de Neurobiología, UNAM, Campus Juriquilla

GR-60 Parthenogenesis: insights from its molecular mechanisms in plants. **Javier Mendiola.** Grupo de Desarrollo Reproductivo y Apomixis. UGA Labotratorio Nacional de Genómica para la Biodiversidad, LANGEBIO

GR-61 Evaluation of the expression and function of histone methyltransferases in hepatocytes of healthy and obese mice. **Paola Berenice Mass Sánchez.** Instituto de Investigaciones Biomédicas. UNAM

GR-62 Identification of germinal and somatic mutations in *BRCA1* and *BRCA2* by next-generation sequencing in patients with breast and ovarian cancer. **Oliver Millan-Catalan.** Laboratorio de Genómica. Instituto Nacional de Cancerología

GR-63 Analysis of the expression of molecules and transcription factors involved in the signaling pathways NF-κB and Adenylate cyclase in the expression of IL-10 in macrophages. **Lourdes Millán Pérez Peña.** Instituto de Ciencias, BUAP

GR-64 Molecular cloning and transient expression of recombinant human PPARγ in HEK293T cells under an inducible Tet-on system. **Sonia Montero Molina.** School of Chemistry, Universidad Autonoma de Nuevo Leon

GR-65 Activity of V2 and V3 promoters from *ST3GAL4* gene under effect of E6 viral oncoprotein from HPV 16. **Monterrosas Santamaría José Ricardo.** Facultad de Ciencias Biológicas, Benemérita Universidad Autónoma de Puebla

GR-66 Protein composition and biological activities of the venom from *Ophryacus sphenophrys.* **Luis Sergio Muñoz Carrillo.** Escuela Nacional de Ciencias Biológicas, IPN

GR-67 Establishment of a cellular model of differentiated human myocytes for the study of the lncRNAs alterations involved in the pathogenesis of myotonic dystrophy type 1 (DM1). **Nadia Mireya Murillo Melo.** Department of Genetics, National Rehabilitation Institute

GR-68 TGF−β1 induced profound methylation changes in fibroblasts, and revealed an unexpected role of Homer1 in idiopathic pulmonary fibrosis. **Miguel Negreros Amaya.** Facultad de Ciencias, UNAM

GR-69 Alteration in the proliferation and migration of colorectal cancer cells derived from the interaction of miR-124 and GPT2. **David Núñez Corona.** Laboratorio de Genómica Funcional del Cáncer (UBIMED)

GR-70 *Debaryomyces hansenii* alternate CUG codon analysis. **Daniel Ochoa Gutiérrez.** Laboratorio de Biología Molecular y Genómica, Facultad de Ciencias, UNAM

GR-71 Interaction of RE1-Silencing Transcription factor with E6 gene of the Human Papillomavirus: a plausible mechanism for E6 transcription regulation. **Carlos Ortuño Pineda.** Universidad Autónoma de Guerrero

GR-72 Analysis of the interaction of YwqL and MutSL during the processing of

deaminated DNA bases in *Bacillus subtilis*. **Adriana Guadalupe Patlán-Vázquez.** Departamento de Biología, Universidad de Guanajuato

GR-73 The role of miRNA regulation during sarcopenia in skeletal muscle from aged female rats. **Gibrán Pedraza-Vázquez.** Universidad Autónoma Metropolitana Iztapalapa. Instituto Nacional de Geriatría

GR-74 Transcriptome analyses of miR-122 knockdown breast cancer cells reveal new insights into molecular determinants of resistance to radiotherapy. **Isidro Xavier Pérez Añorve.** Metropolitan Autonomous University, Cuajimalpa Unit

GR-75 Role of ZNF365 in Pulmonary Fibrosis. **Juan Manuel Pérez Huacuja Urista.** Facultad de Ciencias, UNAM

GR-76 Transformation of *Metarhizium guizhouense* mediated by *Agrobacterium tumefaciens*. **Iván Horacio Piña-Torres.** Department of Biology, Division of Natural and Exact Sciences, University of Guanajuato

GR-77 Finding insights on telomere dynamics in telomerase negative strains of *Ustilago maydis*. **Carmen María Posadas Gutiérrez.** Centro de Investigaciones en Ciencias Microbiológicas. BUAP

GR-78 Fungal metabolities as putative epi-modulators: A case study with BET bromodomain BRD4. **Fernando D. Prieto-Martínez.** Facultad de Química. UNAM

GR-79 Identification of mycorrhizal symbiosis specific autophagy genes under TOR signal disruption in common bean. **Elsa-Herminia Quezada Rodríguez.** Ciencias Agrogenómicas, Escuela Nacional de Estudios Superiores León. UNAM

GR-80 Genetic and physiological interactions between the mitochondrial protein Slm35 and the components of the autophagy machinery. **Josué M.J. Ramírez-Reyes**. Departamento de Genética Molecular. Instituto de Fisiología Celular, UNAM

GR-81 The role of the histone deacetylases in the morphology and virulence of the fungus *Macrophomina phaseolina* (Tassi Goid). **Ramos-García, Víctor Hugo.** Centro de Biotecnología Genómica - IPN

GR-82 Isolation and Expression Levels of SRY-box 9 (SOX9) in Harderian Glands of Syrian Hamster (*Mesocricetus auratus***). Luis Ramos Tavera.** Department of Reproductive Biology, Instituto Nacional de Ciencias Médicas y Nutrición

GR-83 Mutation Analysis of Aldo-Keto Reductases (*AKR1C2/4*) and *HSD17B6* in Subjects 46,XY with Nonsyndromic Hypospadias. **Luis Ramos Tavera.** Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán

GR-84 Transcriptional network evolution underlying biofilm formation in *Candida maltose*. **Aníbal Uriel Reyes Mérida.** Departamento de Ingeniería Genética, CINVESTAV – Irapuato

GR-85 Probing the phosphoryl-group transfer routes in the ArcB dimer. **Rodríguez Rangel C.** Departamento de Genética Molecular, Instituto de Fisiología Celular, UNAM

GR-86 Characterization of the promoter of *MYO1G* and *MYO1F* human: Gene regulation of basal transcription. **Rosa Isela Rodríguez Téllez.** Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, IPN. Hospital Infantil de México

GR-87 Search for genes involved in degradation of Benzo[a]pyrene in *Bacillus*

licheniformis M2-7. **Augusto Rojas-Aparicio.** Nucleic Acid and Protein Laboratory, Autonomous University of Guerrero

IMMUNOLOGY & PARASITOLOGY II

IP-17 Effect of blocking oestrogen receptors on the IFN-γ and IL-10 mRNA expression in the brain of mice infected with *P. berghei* ANKA. **Teresita de Jesús Nolasco-Pérez**. Laboratorio de Inmunología Molecular, Facultad de Estudios Superiores Zaragoza, UNAM

IP-18 Immunomodulatory effects of thionin Thi2.1 from *Arabidopsis thaliana* on bovine mammary epithelial cells. **María T. Arceo-Martínez**. Universidad Michoacana

IP-19 Biochemical characterization of allergens isolated from different sources for skin tests. **Aranxa Zu Ortiz Pacheco**. Universidad Politécnica del Estado de Morelos

IP-20 Cytokine profile in serum and bronchoalveolar lavage from patients with primary Sjögren's Syndrome. **Daniel Paz Gómez**. Instituto Nacional de Enfermedades Respiratorias "Ismael Cosío Villegas". Laboratorio de Enfermedades Reumáticas

IP-21 CD13 mediates phagocytosis in human neutrophils. **Gloria Erandi Pérez Figueroa**. Departamento de Inmunología, Instituto de Investigaciones Biomédicas, UNAM

IP-22 Participation of neutral sphingomyelinases to *in vitro* virulence of *Entamoeba histolytica*. **Fátima Ramírez-Montiel**. Departamento de Biología y Departamento de Farmacia. División de Ciencias Naturales y Exactas. Universidad de Guanajuato

IP-23 Cloning and expression of Cathepsin-B from Fasciola hepatica in *Escherichia coli*. **Miguel Ángel Ramírez Valdivia**. Universidad Politécnica del Estado de Morelos

IP-24 Exploration of innate immune responses of Northern elephant seal pups throughout their fasting period. **Nami Morales-Duran**. Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro

IP-25 Characterization of a mutant of the enzyme trans-sialidase from *Trypanosoma cruzi* (TcTS) and its potential for a vaccine development against chagas disease. **Juana Elizabeth Reyes Martínez**. Ciencias Naturales y Exactas. Universidad de Guanajuato

IP-26 The calcium homeostasis of the Golgi apparatus could be regulated by an SPCA in *Entamoeba histolytica*. **Andrés Salas Casas**. Instituto de Ciencias de la Salud. Universidad Autónoma del Estado de Hidalgo

IP-27 Suramin Evokes Two Effects on Human P2X Receptors of Macrophages. **Eydie Mariela Vargas-Martínez**. Instituto Potosino de Investigación Científica y Tecnológica, A.C.

IP-28 Effect of three different organic compounds on the cytotoxicity of Trichomonas vaginalis toward HeLa cells. **Laura Isabel Vázquez-Carrillo**. Posgrado en Ciencias Genómicas, Universidad Autónoma de la Ciudad de México

IP-29 Gut Mucopolysaccharide Levels in Mice Treated with Bovine Lactoferrin. **Vega-Bautista Alan**. Departamento de Sistemas Biológicos, UAM-Xochimilco

IP-30 Production, characterization and epitope mapping of a novel anti-N-

truncated/pyroglutamate-modified AβN3(pE) peptide. **David Vladimir Venegas Jerónimo**. Instituto de Investigaciones Biomédicas, UNAM

IP-31 Exposure to an Enriched Environment Attenuates Mouse Experimental Colitis. **Tomás Villaseñor Toledo.** Laboratorio de Neuroinmunobiología, Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología, UNAM

MEDICINE HEALTH & NUTRITION III

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MH-57 Association between insulin resistance and anthropometric, physiologic and metabolic risk factors in scholars from the state of Queretaro. **Alan M. Juárez-Ramírez**. School of Medicine. Autonomous University of Queretaro

MH-58 Effect of curcumin on the proteins glycation and ppar expression protein in heart of mice fed with high fructose content. **María Cristina León-García**. Dpt. of Medical Sciences, University of Guanajuato

MH-59 Expression of ITGβ1 and LAMC1 in cervical samples from patients with HPV infection, Intraepithelial Squamous Lesion and cervical cáncer. **Alejandro Linares-Martínez**. Ciencias Químico Biólogo Parasitólogo, Universidad Autónoma de Guerrero

MH-60 Evaluation of the correlation between renal diseases and obesity. **Jarumi de los Ángeles López Flores**. Universidad Juárez Autónoma de Tabasco

MH-61 Antiproliferative, necrotic and apoptotic activity of the glycoconjugates Diosgenin-2-acetamido-2-deoxy-beta-D-glucopyranoside (MF-10) and Diosgenyl 2-amino-2-deoxy-beta-D-glucopyranoside hydrochloride (MF-11) In Vitro. **Hugo López Muñoz**. FES Zaragoza, UNAM

MH-62 Antiproliferative effect of naringenin with de coexposition with bisphenol A in colon cancer cells. **Sara Julietta Lozano Herrera**. Facultad de Medicina. Universidad Autónoma de Querétaro

MH-63 Molecular interactions between human osteosarcoma biomarkers and cancer treatment drugs. **Salvador Augusto Macías-Sánchez**. Facultad de Medicina, Universidad Autónoma del Estado de Morelos

MH-64 Effects of *V. meridionale* on insulin resistance and HDL function markers in women with metabolic syndrome: a randomized, placebo-controlled trial. **Marín-Echeverri C**. Escuela de Microbiología. Universidad de Antioquia

MH-65 MMP-9 in presence of nicotinamide in breast cancer cells. **María Guadalupe Martínez Hernández**. Morphology and Function Unity, Faculty of Professional Studies Iztacala, UNAM

MH-66 IGF / IGFR complex expression in the MDA MB 231 tumor line: role of ERβ activation. **Max Alejandro Maximino Rojas**. Instituto de Fisiología, Benemérita Universidad Autónoma de Puebla

MH-67 Magnetic signaling with modified nanoparticles. **Ixaura Celeste Medina-Medina**. Laboratorio de Interacciones Biomoleculares y Cáncer. Instituto de Física, UASLP

MH-68 miRNA expression profiling of liver cancer cells-derived extracellular vesicles: an approach for hepatocellular carcinoma diagnostic. **Francisco Alfredo Molina**

Pelayo. Universidad de Colima

MH-69 *In vitro* evaluation of a new bioactive and biphasic implant with potential application in osteochondral tissue engineering. **Nidia Karina Moncada-Saucedo**. Departamento de Bioquímica. Facultad de Medicina. UANL

MH-70 microRNAs implications in the intracellular calcium dynamics regulation in hepatocellular carcinoma. **Jesús Monroy Rodríguez**. Centro Universitario de Investigaciones Biomédicas, Universidad de Colima

MH-71 Effect of rice bran on oxidative stress in older adults with cognitive impairment. **Alejandro Morales Aparicio**. Escuela Superior de Medicina, IPN

MH-72 Rhythmic Changes in Oxidative Stress Indicators of Ovarian Tissues in Adult Rats Related to Expression on Dopaminergic Receptor Type 1 (RDA1) and DARPP-32 in Different Steps of Estral Cycle. **José Luis Morán Perales**. Benemérita Universidad Autónoma de Puebla, Instituto de Ciencias

MH-73 Design and evaluation of selective cell penetrating peptides in mammalian cells. **Rafael Ubaldo Morán Torres**. Instituto de Fisiología Celular, UNAM

MH-74 Study of structure, function, and relationship of platelet´s receptors and membrane bilayers in aterosclerosis. **Armando Ocampo del Valle**. Universidad Autónoma del Estado de Morelos, Facultad de Medicina

MH-75 Route of food allergens administration is critical for the sensitization of BALB/c mice. **Noé Ontiveros Apodaca**, Departamento de Ciencias Químico Biológicas y Agropecuarias, Universidad de Sonora.

MH-76 Caffeine inhibits migration and invasion in MDA-MB-231 breast cancer cell line. **Mario Israel Oregel Cortez.** Facultad de Medicina, Universidad Autónoma de Baja California

MH-77 Effect of curcumin on PPARα and MLYCD expression in liver of high fructose diet-treated mice. **Luz A. Ortega-Hernández**. División de Ciencias de la Salud, Campus León, Universidad de Guanajuato

MH-78 CETPI: a new protein involved in the pathophysiology of sepsis and septic shock. **Eréndira Guadalupe Pérez Hernández**. Instituto de Fisiología Celular, UNAM

MH-79 Bovine cartilage decellularized matrix improves chondrogenic differentiation and Young's modulus of Silk fibroin scaffolds. **Vanessa Pérez-Silos**. Departamento de Bioquímica. Facultad de Medicina. Universidad Autónoma de Nuevo León

MH-80 High HPV prevalence and its effects on pro-inflammatory cytokine expression in semen of Mexican patients. **Elvia Pérez Soto**. Facultad de Medicina, Autónoma del Estado de México

MH-81 Polymorphism detection in genes associated to isoniazid metabolism for dosage adjustment in San Luis Potosi. **Pettet-Ruiz Guillermo**. Instituto Potosino de Investigación Científica y Tecnológica

MH-82 CLIC5 subcellular localization and participation in Hepatocellular Carcinoma. **Carolina Piña Vázquez**. CINVESTAV IPN

MH-83 YY1 negatively regulates the XAF1 gene expression. **Miguel Angel Quevedo-Castillo**. Facultad de Medicina, UNAM

MH-84 The effect of administrating an extract from pitaya juice rich in betalains on cisplatin induced nephrotoxicity and cellular damage, *in vivo* and *in vitro*. **Yadira Ramírez-Rodríguez**. Instituto Potosino de Investigación Científica y Tecnológica

MH-85 Polymorphism rs2295490 in the TRIB3 gene and its relationship with lipid metabolism in patients with type 2 diabetes. **Xochilth Natividad Ramos Deloya**. Facultad de Ciencias Químico Biológicas. Universidad Autónoma de Guerrero

MICROBIOLOGY & VIROLOGY III

MV-50 Determination of factors that define the tropism of astrovirus in established cell lines. **Guzmán-Ruiz Leticia.** Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, UNAM

MV-51 Isolation and characterization of bacteria resistant to cobalt. **César Díaz Pérez.** Campus Celaya-Salvatierra. Universidad de Guanajuato

MV-52 Assessing squash root microbiome along a historical aridity gradient. **Cristóbal Hernández Álvarez.** Departamento de Biología Celular, Facultad de Ciencias

MV-53 Effect of antimicrobial activity of hexanic and ethanolic extracts of Lippia graveolens against the *Salmonella enterica*. **María Susana Hernández-Hernández.** Universidad Tecnológica de Morelia

MV-54 Determination of the regulatory mechanism of the Toxin-Antitoxin system of plasmid pUM505. **Karen Cecilia Hernández Ramírez**. Instituto de investigaciones Químico Biológicas

MV-55 Influenzavirus A(H1N1)pdm09 enhances the adhesion of *A. fumigatus* resting conidia to the alveolar epithelial cell line A549. **Fernando Hernández-Sánchez**. Instituto Nacional de Enfermedades Respiratorias

MV-56 Isolation and characterization of bacteria with plant growth promotion rizobacteria (PGPR) associated to native maize of a region of the state of Guerrero. **Zaida Asunción Hernández-Martínez.** Instituto de Ecología A.C.

MV-57 Search, amplification and cloning of the gene *csr*A in *Bacillus licheniformis* M2-7. **Juanita Jacobo Valerio**. Laboratory of Biotechnology, Autonomous University of Guerrero

MV-58 Assessment of protein fractions culture supernatant of Mycobacterium bovis in interferon gamma release assays by ELIspot. **Laura Jaramillo Meza.** CENID-Microbiología Animal INIFAP

MV-59 Evaluation of the effect of trypsin on the biofilm-forming capabilities of commensal and infective isolates of *Staphylococcus epidermidis***. Sergio Martínez-García**. Escuela Nacional de Ciencias Biológicas, IPN

MV-60 Characterization of a novel ciprofloxacin resistance protein plasmid encoded, CrpP. **Víctor M. Chávez-Jacobo**. Instituto de Investigaciones Químico-Biológicas, UMSNH

MV-61 Partial characterization of a lipoprotein from coliphage mEp021 involved in Superinfection Exclusion. **Martínez Soto Carlos E.** Departamento de Genética y Biología Molecular. CINVESTAV-Unidad Zacatenco

MV-62 Study of essential genes in *Pseudomonas aeruginosa*. **Enrique Martínez-Carranza.** Instituto de Investigaciones Biomédicas, UNAM

MV-63 Obtention of a ctpF knockout mutant in Mycobacterium tuberculosis: a potential target of attenuation. **Milena Maya Hoyos.** Departamento de Química. Facultad de Ciencias. Universidad Nacional de Colombia

MV-64 Biological traps to recruit plant associated microbiomes, in multiple types of soils. **Gerardo Mejía.** Departamento de Biología Celular, Facultad de Ciencias, UNAM

MV-65 Filamentous Fungi Cell Wall PIR Proteins: Diversity, Function and Application. **Paul Montaño-Silva**. Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco

MV-66 Epinephrine and norepinephrine induce Actinobacillus seminis biofilm dispersion. **Juan Fernando Montes-García.** Facultad de Estudios Superiores Iztacala, UNAM

MV-67 Characterization functional of riboswitches T-box in Firmicutes and the other gram-postive bacteria. **Nataly Morales Galeana.** Instituto de Biotecnología, UNAM

MV-68 Regulation of E2348C_1013 by GrlA in enteropathogenic *Escherichia coli.* **Álvaro Damián Morales Ibarra.** Instituto de Biotecnología, UNAM

MV-69 Potential of colonization from fungal endophyte isolated from *Eichhornia crassipes* on bean plants. **Adán Topiltzin Morales-Vargas**. Universidad de Guanajuato, Campus Celaya-Salvatierra

MV-70 Characterization of plant growth promoting rhizobacterium isolated from saline environments of the Chihuahuan Desert. **Claudia Estefania Navarro Muñoz.** Facultad de Ciencias Biológicas, Universidad Juárez del Estado de Durango

MV-71 Lambda phage display as a model for a ZIKV vaccine. **Negrete-Méndez, Honorio.** Departamento de Genética y Biología Molecular. CINVESTAV-Unidad Zacatenco

MV-72 Analysis of the regulation and function of chaperone-usher fimbrial operons in *Citrobacter rodentium*. **Ortiz-Jiménez Stephanie**. Instituto de Biotecnología, UNAM

MV-73 Human Papillomavirus prevalence in oral cavity from University population. **Nidia Gary Pazos Salazar.** Facultad de Ciencias Químicas, BUAP

MV-74 Rotaviruses associate with extracellular vesicles in MA104 cells. **Arianna Pérez Delgado.** Instituto de Biotecnología, UNAM

BIOCHEMISTRY AND PLANT MOLECULAR BIOLOGY

P-1 Diversification of tRNA genes through duplication in *Cucurbita*. **Xitlali Aguirre Dugua**. Facultad de Estudios Superiores Iztacala, UNAM

P-2 Real-Time Monitoring of *Nicotiana benthamiana* Volatile Response to Microbial Scents by Low-Temperature Plasma Mass Spectrometry. **Raúl Alcalde Vázquez**. Centro de Investigación y de Estudios Avanzados del IPN, Unidad Irapuato

P-3 Subcellular Localization of long non-coding RNA Using Modified Two Component System in Plants. **Jaime Aportela Cortez**. Instituto de Biotecnología, UNAM

P-4 Cold tolerance and RNA binding activity of the AtGRDP2 protein in *Arabidopsis thaliana*. **Saraí Castro Bustos**. División de Biología Molecular, IPICYT, A.C.

P-5 Evaluation of gene expression and translational status in the silencing line amiR: ADC-L2 of *Arabidopsis thaliana* using polysome profiles and detection of transcripts. **Montserrat Chacón-Flores**. División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica, A.C.

P-6 Generation of scientific and technological strategies with a multidisciplinary and interinstitutional approach to face the threat represented by exotic ambrosia beetles to the agricultural and forestry sectors of Mexico. **José A. Guerrero-Analco**. Instituto de Ecología A.C.

P-7 Factors that regulate SPATULA (SPT) expression in *Arabidopsis thaliana.* **Angela Guadalupe Juárez-Corona**. Unidad de Genómica Avanzada (LANGEBIO), CINVESTAV-IPN

P-8 Cracking the mysteries of zygotic embryogenesis in avocado by systems biology. **Janet Juarez-Escobar**. Red de Estudios Moleculares Avanzados, Instituto de Ecología A.C.

P-9 The effect of synthetic microbial rhizosphere community on the growth of pioneer plants, in mine tailings. **Arely Lechuga-Jiménez**. Departamento de Biología Celular Facultad de Ciencias, UNAM

P-10 Identification of candidate genes regulated by ATX1 and involved in root development. **Selene Napsucialy Mendivil**. Departamento de Biología Molecular de Plantas, Instituto de Biotecnología, UNAM

P-11 Lead shortening of primary root share mechanism with phosphate starvation and is independent of the STOP1 pathway. **Ortiz-Luevano Ricardo**. Unidad Académica de Ciencias Biológicas Universidad Autónoma de Zacatecas

P-12 Absence of BYPASS1 signal aborts the arbuscular mycorrhizal invasion in *Phaseolus vulgaris.* **Manoj-Kumar Arthikala.** Ciencias Agrogenómicas, Escuela Nacional de Estudios Superiores, Unidad León. UNAM

CELL BIOLOGY

CB-1 Proteins implicated during the formation and degradation of Lipid droplets in *Ustilago maydis*. **Minerva Georgina Araiza Villanueva**. Escuela Nacional de Ciencias Biológicas, IPN

CB-2 Changes of Acrosomal pH During Human Sperm Capacitation. **Gabriela Carrasquel Martínez**. Instituto de Biotecnología,UNAM

CB-3 Actin cytoskeleton dynamics during human sperm capacitation. **Valeria Castillo-Viveros**. Instituto de Biotecnología, UNAM

CB-4 Image-based flow cytometry as a tool to study rheotaxis in human spermatozoa. **Mariana Beatriz Olivares Urbano.** Instituto de Biotecnología, UNAM

CB-5 Expression of Septins 1 and 2 during the Oogenesis of *Aedes aegypti*. **José Ángel Rubio Miranda**. CINVESTAV IPN.

CB-6 A predicted cargo adaptor, CNI, plays an important role in traffic of specific essential material for the growth of *Neurospora crassa*. **Luis Enrique Sastré-Velásquez**. Departamento de Microbiología. CICESE

BASIC BIOCHEMISTRY IV

B-88 Biochemical caracterization for the enzyme *Kl*Bat1, a branched-chain amino acid transaminase in *Kluyveromyces lactis*. **Escudero-Reyes J. Raul**. Bioquímica y Biología Estructural, Instituto de Fisiología Celular, UNAM

B-89 Changes in the transcription of autophagy genes and proteases in aged cells of yeast. **René Abraham Mejía Cahuantzi**. Instituto de Ciencias. Benemérita Universidad Autonóma de Puebla

B-90 Modification in the mRNA level of transporter coding genes in aged cells of yeast. **Nora Hilda Rosas Murrieta.** Centro de Química. ICUAP. Benemérita Universidad Autonóma de Puebla

B-91 Betaine Aldehyde Dehydrogenase is regulated during WSSV infection in white shrimp. **Jesús Alfredo Rosas Rodríguez.** Universidad Estatal de Sonora

B-92 Characterization of the human solute carrier SLC16A11 and its sequence variant present in Type 2 Diabetes. **Hilda Sánchez Vidal**. Department of Food Science and Technology, Instituto Nacional de Ciencias Médicas y Nutrición

B-93 Structural characterization of the heavy chain of dynein. **Santiago Sánchez Saúl.** Lab. 2. CINVESTAV Zacatenco

B-94 Mitochondrial permeability transition is involved in silica nanoparticle-induces cardiac injury through oxidative stress. **Christian Silva-Platas.** Escuela Nacional de Medicina y Ciencias de la Salud, Tecnológico de Monterrey

B-95 NMR-based metabolomic analysis on the seasonal variation of *Ternstroemia pringlei* (Rose) Standl. **Alexis Uriel Soto- Díaz.** Centro de Investigación en Biotecnología, UAEM

B-96 Effect and study of inhibitors of the enzymes DNA topoisomerases I and II on *Candida* spp. **Tagle-Olmedo Tania.** Laboratorio de Biología Molecular de Bacterias y Levaduras. Escuela Nacional de Ciencias Biológicas, IPN

B-97 ARP2; a determinant molecule in apoptosis of tumoral cells, new implications in atherosclerosis. **Juana Virginia Tapia-Vieyra**. Instituto de Fisiología Celular, UNAM

B-98 Does the NaStEP-NaSIPP interaction in mitochondria trigger programmed cell death in Nicotiana pollen tubes? **Edgar Nájera Torres.** Departamento de Bioquímica. Facultad de Química, UNAM

B-99 Characterization of super-complex with $NADP^+$-isocitrate dehydrogenase activity in mitochondria from human placenta. **Viviana Urban-Sosa.** Departamento de Bioquímica, Facultad de Medicina, UNAM

B-100 Biochemical, biophysical and structural characterization of isoniazid resistance KatG variants from *Mycobacterium tuberculosis*. **Brenda Georgina Uribe Vázquez.** Cellular Engineering and Biocatalysis Department, Biotechnology Institute, UNAM

B-101 *Cis*-regulatory evolution leads to the expression of the *phoH* ancestral gene in conditions relevant for *Salmonella* virulence. **Marcos Antonio Valdespino Díaz.** Instituto de Biotecnología, UNAM

B-102 Biochemical and physiological characterization of plasma membrane H+-ATPases PMA1 and PMA2 from corn smut basidiomycete *Ustilago maydis*. **Melissa Vázquez-**

Carrada. Escuela Nacional de Ciencias Biológicas, IPN

B-103 Analysis of the control of the trypanothione synthesis in *Trypanosoma cruzi*. **Citlali Vázquez.** Instituto Nacional de Cardiología Ignacio Chávez

B-104 Biochemical characterization of the thermolabile lecithin dependent hemolysin of *Vibrio parahaemolyticus*. **Luis Enrique Vazquez Morado.** Universidad de Sonora

B-105 Integrity, activity and stoichiometry of the respiratory supercomplexes of *Ustilago maydis*. **Héctor Vázquez-Meza**, Deyamira Matuz-Mares, Juan Pablo Pardo. Department of Biochemistry, Faculty of Medicine, UNAM

B-106 Structural stability of the glucose-6-phosphate dehydrogenase of *Pseudomonas aeruginosa* provided by its substrate. **Roberto Velasco-García.** Facultad de Estudios Superiores Iztacala. UNAM

B-107 Layered Double Hydroxides (LDH) for the immobilization by metal affinity of genetic engineered enzymes for the development of biosensors. **Gilberto Velázquez-Juárez.** Departamento de Química. CUCEI. Universidad de Guadalajara

B-108 PGC-1 and perilipin during physiological cardiac hypertrophy induced by pregnancy. **Denisse Verduzco-Ávila.** Ciencias Químico-Biológicas y Agropecuarias. Universidad de Sonora

B-109 A suppressor mutation of Mss51 restores Cox1 synthesis. **Cristian Alberto Vergara Gerónimo.** Genética Molecular, Instituto de Fisiología Celular, UNAM

B-110 Kinetic and thermodynamic characterization of the folding of the lysinearginine-ornithine binding protein (LAO) and its individual domains. **Jesús Renán Vergara Gutiérrez.** Departamento de Bioquímica, Facultad de Medicina, UNAM

B-111 Mitigation of oxidative stress in neurodegenerative processes by the activation of aldehyde dehydrogenases. **Belem Yoval Sánchez.** Instituto Nacional de Cardiología “Ignacio Chávez”

BIOTECHNOLOGY IV

BT-109 Identification of pigments in strains of Pycnoporus. **Leonardo Romo Patiño.** Facultad de Farmacia, Universidad Autónoma del Estado de Morelos

BT-110 Design of a chromogenic culture medium based on Nejayote Water for the identification of *Candida albicans* involved in Vaginal Candidiasis. **Tayde Sepori Salgado Hernández.** Centro de Investigación en Biotecnología, UAEM

BT-111 Physiological and molecular characterization of *Saccharomyces cerevisiae* strains for industrial Wine production. **Cindy Zuleyka Sánchez Arias.** Departamento de Biología. División de Ciencias Naturales y Exactas. Universidad de Guanajuato

BT-112 Endophytes non- rhizobiales isolated from nodules of *Mimosa pudica* with biotechnological potential. **Ricardo Sánchez Cruz.** CEIB. Universidad Autónoma del Estado de Morelos

BT-113 Molecular characterization of fructansucrase of *Clavibacter michiganensis* subsp *michiganensis*. **Azalia Sánchez Cruz.** Universidad Politécnica del Estado de Morelos

BT-114 Preparation of nanostructures with controlled architecture by the self-assembly of designed DNA and proteins. **Eddie Guillermo Sánchez-Rueda.** Instituto de

Química UNAM

BT-115 Development of a nanocomposite with antibiofilm activity and permissive for delivery of Mesenchymal Stem Cells. **Sánchez-Sánchez, Roberto.** Unidad de Ingeniería de Tejidos Terapia Celular y Medicina Regenerativa, INR

BT-116 Generating a diagnostic method for differentiation between Zika and Chikungunya virus by PCR, using samples Tabasco State. **Santos Fernández Ingrid Araceli.** Universidad Juárez Autónoma de Tabasco

BT-117 Evaluation and microbiological determination, organoleptic and physicochemical of beer production with wild yeast. **Álvarez Quiroz Ernesto.** Facultad de Ciencias Biológicas. ICUAP BUAP

BT-118 Arbuscular mycorrhizal symbiosis, growth and photochemical activity are affected by the phosphate concentration in *Stevia rebaudiana*. **Luis Gerardo Sarmiento López.** CEPROBI IPN

BT-119 Sub-cloning and heterologous expression of Mnn1 enzyme from *S. cerevisiae* in *E. coli*. **Esteban Francisco Serrano.** División de Ciencias Naturales y Exactas. Universidad de Guanajuato

BT-120 Deciphering the role of bean *RALF*, *FER* and *RIPK* genes in symbiosis with rhizobia. **Jorge Solís-Miranda**. Department of Plant Molecular Biology, Institute of Biotechnology, UNAM

BT-121 Discovery of two bacterial type III polyketides with potential bioactive capacity from marine ecological niches. **Arianna Soto-Hernández.** Universidad de Colima

BT-122 Partial characterization of *Scolopendra viridis* Say and *S. polymorpha* venom proteases. **Erika Duarte-Elguea.** Centro de Investigación en Biotecnología, UAEM

BT-123 Activity of extracellular laccases of *Humpherella coffeatum* grown on different substrates. **Maura Téllez Téllez.** Centro de Investigaciones Biológicas. UAEM

BT-124 Growth of *Pleurotus ostreatus* in airlift reactor and stirred tank for laccase production. **Maura Téllez Téllez.** Maestria en Biotecnología y Manejo de Recursos Naturales, Universidad Autónoma de Tlaxcala

BT-125 Entomochemicals from *Pterophylla beltrani* as bioactive compounds for food industry**. Jorge Ariel Torres-Castillo.** Instituto de Ecología Aplicada. Universidad Autónoma Tamaulipas

BT-126 Characterization of cadB1 a new gene involved in the degradation pathway of chloranilic acid in *Herbaspirillum* sp. strain TQ07. **Luis Gerardo Treviño Quintanilla.** Universidad Politécnica del Estado de Morelos

BT-127 Expression of Loos1 from fungus Bjerkandera adusta in plants of *Arabidopsis thaliana* regulated by an inducible promoter to develop an auto-pretreatment protocol. **Maria Magdalena Urzua-Abad.** Centro de Investigación en Biotecnología. UAEM

BT-128 Efficient multiplication of *Vaccinium corymbosum* in temporary immersion systems (SIT). **Miriam Isabel Vargas Avila.** Unidad Profesional Interdisciplinaria de Ingeniería. Campus Guanajuato. IPN

BT-129 *Pyr4*, a new selectable auxotrophic genetic marker in *Ustilago maydis*. **Alma Delia Vega Jiménez**. Laboratorio de Microbiología Molecular. Facultad de Ciencias

Naturales, Universidad Autónoma De Querétaro

BT-130 Interaction studies at the molecular level protein-peptide, using calmodulin as a molecular target. **Isabel Velázquez-López.** Departamento de Bioquímica, Facultad de Medicina, UNAM

BT-131 A fluorescent assay for the detection of alkyl glucosides and other non-ionic surfactants. **Wendy Xolalpa Villanueva**. Instituto de Biotecnología, UNAM

BT-132 Influence of bacteria in the digestive system of the worm *Eisenia Foetida* in the degradation of polychlorinated biphenyls. **Adalberto Zenteno Rojas.** Instituto Tecnológico de Tuxtla-Gutiérrez

BT-133 Incorporation of ORF2 from Porcine Circovirus Type 2 into genetically encoded particles by a self-aggregating peptide and its use as subunit vaccine. **Jesús Zepeda-Cervantes.** Instituto de Fisiología Celular, UNAM

BT-134 Identification and characterization of the chitinases and glucanases produced by *Wickerhamomyces anomalus* and *Trichoderma harzianum* against crops phytopathogens. **Zepeda Giraud Luis Fernando.** ENCB. IPN

REACTIVE OXYGEN SPECIES II

ROS-15 Low-intensity and long-term training prevents sarcopenic obesity in female Wistar rats. **Beatriz Mena-Montes.** Ciencias de la Salud, UAM Iztapalapa

ROS-16 The cardioprotective effect of sulforaphane is mediated trough an Nrf-2 independent pathway. **Marcos Ostolga Chavarría**. Biomedicina Cardiovascular. Instituto Nacional de Cardiología

ROS-17 Differential effects of carbohydrates and fatty acids on oxidative stress and mitochondrial alteration in a hypothalamic cellular line. **Itzel Pérez Ayala.** Facultad de Química, Universidad Autónoma de Querétaro

ROS-18 Effect of 17-β estradiol and progesterone on the evolution of Myocardial Infarction. **Diana Ramírez Hernández**. Facultad de Estudios Superiores Cuautitlán, UNAM

ROS-19 Moringa oleifera extract attenuates alloxan-induced metabolic changes through suppressing the iNOS expression and modulating eNOS activity in heart and liver of rats. **Cristina Ramos Olivas.** Universidad Juárez del Estado de Durango

ROS-20 Synthesis and characterization of antioxidant compounds and inhibitors of myeloperoxidase derived from cinnamic acid. **Astrid Mayleth Rivera Antonio.** Interdisciplinary Professional Unit of Biotechnology, IPN

ROS-21 Fatty acids promoted oxidative stress in *Yarrowia lipolytica* cells. **Sandra Lucía Rodríguez-Teniente.** Facultad de Salud Pública y Nutrición, Universidad Autónoma de Nuevo León

ROS-22 Association of telomere length, oxidative stress and frailty in an elder population. **José Darío Martínez-Ezquerro**. Unidad de Investigación Médica en Genética Humana. Centro Médico Nacional Siglo XXI, IMSS

ROS-23 Does the antioxidant system and ABA are involved in aluminum tolerance during

the first hours of treatment in *Fagopyrum esculentum* seedlings? **Ana Violeta Salazar-Chavarría.** Instituto de Ecología, UNAM

ROS-24 Participation of Iba57p in the [2Fe-2S] cluster assembly of the Rip1 subunit into the cytochrome *bc1* complex from *Saccharomyces cerevisiae*. **Luis Alberto Sánchez Briones.** IIQB Universidad Michoacana

ROS-25 Sulforaphane effects on nerve conduction velocity and brain cortex and hippocampus redox state of old male and female Wistar rats. **Roberto Santín Márquez.** Ciencias de la Salud, UAM Unidad Iztapalapa

ROS-26 Effect of exposure to low doses of ozone on the expression of IL-17A during the process of progressive neurodegeneration in the hippocampus of rats. **Helena Solleiro-Villavicencio.** Facultad de Medicina, UNAM

ROS-27 Malondialdehyde and carbonyl levels in Wistar rats treated with streptozotocin and sugar water in neonatal age. **Sergio Trinidad Rodríguez.** Facultad de Ciencias Químico Biológicas. Universidad Autónoma de Guerrero

ROS-28 Evaluation of the enzymatic activity of catalase in a model of cancer treated with extracts of *Crotalaria retusa*. **José Roberto Velázquez Murillo.** Facultad de Estudios Superiores Zaragoza, UNAM

GENETICS, EPIGENETICS AND GENETIC REGULATION IV

GR-88 Nuclear organization of breast cancer oncogenes: a new approach using CRISPR-dCas9 technology. **Pablo Antonio Rojas Reyes.** Instituto de Investigaciones Biomédicas, UNAM

GR-89 Study of light response in *Metarhizium* spp. **Adriana García Tapia.** Universidad de Guanajuato

GR-90 Characterization of differentially expressed long non-coding RNAs during the interaction between adipose-derived stem cells and cervical cancer cells. **Victor Hugo Rosales-Gallegos.** Instituto Nacional de Medicina Genómica

GR-91 Functional characterization of mutations identified in the LMNA gene associated with dilated cardiomyopathy in Mexican patients. **Sandra Rosas-Madrigal.** Instituto Nacional de Medicina Genómica

GR-92 Genetic edition in filamentous fungi mediated by CRISPR: *Sclerotium cepivorum* Berk and *Trichoderma atroviride*. **Luis Mauricio Salazar García.** Departamento de Biología. Universidad de Guanajuato

GR-93 Effects of CBFB inhibition by CRISPR-Cas in a breast cancer cell line. **Ixchel Maritrini Salgado Carranza.** Instituto Nacional de Medicina Genómica

GR-94 HOTAIR knockdown inhibits Wnt pathway by re-expression of its negative regulators. **Eric Genaro Salmerón Bárcenas.** Laboratorio de Epigenética del Cáncer. Universidad Autónoma de Guerrero

GR-95 Analysis of miRNAs and their relationship with molecular pathways of signaling to apoptosis, autophagy and inflammation, during the process sarcopenic in quadriceps femoral muscle tissue. **Jorge Sánchez Cedillo.** Hospital General de México

GR-96 Phosphate deficiency negatively affects early steps of the symbiosis between

common bean and rhizobia. **María del Socorro Sánchez-Correa.** Laboratorio de Genómica Funcional de Leguminosas. FES Iztacala, UNAM

GR-97 LINC00052 roles in MCF-7 breast cancer cells, a migration inhibitor. **José Manuel Sánchez López.** Instituto Nacional de Medicina Genómica

GR-98 MIR-7 and its target genes expression in breast cancer cell lines. **David Sánchez Marín.** Unidad de Biomedicina, Facultad de Estudios Superiores Iztacala, UNAM

GR-99 In silico identification of the putative gene of the RNA subunit of telomerase and analysis of its disrupted mutants in *Ustilago maydis*. **Juan Antonio Sanpedro Luna.** Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla

GR-100 *Fabaceae* miR2199 regulates a bHLH transcription factor mRNA in response to water deficit. **Carlos Alfonso Sierra-Sarabia.** Departamento de Biología Molecular de Plantas, Instituto de Biotecnología, UNAM

GR-101 Transcriptional coupling of base excision repair in sporulating *Bacillus subtilis* cells. **Valeria P. Suárez Castro.** Departamento de Biología, Universidad de Guanajuato

GR-102 Pharmacogenomic algorithm for acenocumarol dosing. **Tomás Eduardo Texis Valencia.** Instituto Nacional de Medicina Genómica

GR-103 Relationship between *MCP-1* A-2518G polymorphism and microvascular complications in Mexican patients with type 2 diabetes mellitus. **Amairany Torres Alvarez.** Posgraduate Program in Genomic Sciences, Autonomous University of Mexico City

GR-104 Exploring the epigenetic convergence between tumorigenesis in mammals and seed formation in flowering plants. **Mijael Alejandro Torres Mendoza.** Unidad de Genómica Avanzada. LANGEBIO. CINVESTAV Irapuato

GR-105 Analysis of the photoreversible fluorescent protein iLOV as a new reporter gene to evaluate promoters in the protozoan *Giardia lamblia*. **María Fernanda Torres-Rojas.** Instituto Nacional de Pediatría. Secretaría de Salud

GR-106 Evaluación de la actividad transcripcional de la vía de señalización WNT-β-catenina en líneas celulares de cáncer colorrectal. **Samuel Trujano Camacho.** Facultad de Estudios Superiores Iztacala. UNAM

GR-107 Circulating microRNAs profile in plasma patients with spinocerebellar ataxia type 7 (SCA7). **Claudia Valdez-Vargas.** Laboratory of Genomic Medicine, Department of Genetics, Instituto Nacional de Rehabilitación

GR-108 RNA-seq approach to study seed dormancy and germination in *Cedrela odorata* L. **Miguel Ángel Vallejo Reyna.** Centro Nacional de Investigación Disciplinaria en Conservación y Mejoramiento de Ecosistemas Forestales, INIFAP

GR-109 Analysis of promoter activity of the human gene *RB1* in hepatic cancer cell lines by epigenetic drugs. **Jorge Eduardo Vargas Gómez.** Centro de Investigación Biomédica del Noreste. Instituto Mexicano del Seguro Social

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El Golem, Frankenstein y la secularización de la biología

Antonio Lazcano Araujo

Miembro de El Colegio Nacional
Universidad Nacional Autónoma de México

Together with the pioneering proposals on biological evolution by Lamarck and Buffon, the secular description of living phenomena is one of the most remarkable scientific achievements of the Enlightenment. It represents a major intellectual watershed echoed in Mary Shelley's *Frankenstein,* which can be read as an extraordinary literary testimony of the accelerated development of a materialistic perspective of life. While the clay-based Golem is animated by the religious invocations of the rabbi Judah Loew, Frankenstein's creature is rendered alive by electricity, a purely physical force.

Mary Shelley was a talented, precocious woman that lived in a refined atmosphere in which science, art and social and philosophical issues were constantly addressed. Galvanism had gained many adherents in these circles, as shown by James Graham's electrical medicine, the development of electrochemistry by Humpry Davis, and the experiments on nervous impulses that Alexander von Humboldt performed upon himself. The reputed life-giving properties of electrical currents prompted Lamarck to include them as part of the "subtle fluids" that had animated the first living beings, while Erasmus Darwin, Benjamin Franklin and William Lawrence, who was Percy Shelley's physician, promoted electricity as a secular therapeutic agent.

Not all accepted this radical perspective. The Swedish scientist Jön Jacob Berzelius was convinced that the difficulties with the electrical breakdown of compounds of biological origin evidenced their special nature. Although he was persuaded that animals were machines, he wrote that "the cause of most of the phenomenon in the animal body is so deeply hidden from our understanding that we will never discover it. We call this hidden cause the vital force". Despite his occasional flirtations with materialism, Berzelius did not turn towards electricity to explain the nature of this "vital force", and argued that the separation between organic and inorganic chemistry could not be bridged. In 1827 he wrote that "art cannot combine the elements of inorganic matter in the manner of living nature", but only one year later his friend and former student Wöhler reported the laboratory synthesis of urea, contributing to narrow the gap separating life from the non-living world.

“Secondary Metabolites in Microbial Communities”

Jo Handelsman

Microbial communities drive critical processes of the Earth and its inhabitants. Geochemical nutrient cycling, climate, crop productivity, and animal and human health are all governed by microbial communities. Much attention has been paid recently to the human microbiome—the microbial communities associated with the human body—because of the explosion of knowledge indicating their roles in diseases as diverse as obesity, asthma, and depression. To ultimately manipulate or modify microbiomes to achieve healthy outcomes, we need to understand the basic rules of how microbiomes respond to perturbations and prevent or permit invasion by alien organisms. Building upon the evidence that model systems have spurred fantastic understanding of molecules, cells, and organisms over the last century, our lab designed a model system that is enabling dissection of a microbiome with the same precision that has been applied to gene expression and development. The model community contains three members—*Bacillus cereus*, *Flavobacterium johnsoniae,* and *Pseudomonas koreensis*—all derived from the rhizospheres of field-grown soybean plants. The members interact in numerous ways, most mediated by small molecules that act as signals or inhibit or promote growth of the other members of the community. The three-member model community displays emergent properties, such as biofilm formation, that are not apparent with any of the members individually or in pairs. Genomic and genetic analyses of all three members have the potential to reveal the rules that govern establishment and robustness of this model community.

NAD^+ and the regulation of virulence in the yeast pathogen *Candida glabrata*

Basil Hussain, Shih-Jung Pan, Zhuwei Xu, Elizabeth Hwang-Wong, Brian Green, Nicole Benoit, Carlos Gomez and **Brendan Cormack**
Johns Hopkins University School of Medicine. Department of Molecular Biology and Genetics
725 N. Wolfe St. Baltimore, MD 21205

Unlike *Saccharomyces cerevisiae,* which can synthesize NAD^+ *de novo* from tryptophan, or salvage it from precursors such as nicotinic acid (NA), nicotinamide ribose (NR) and nicotinamide (NAM), *Candida glabrata* has lost the genes for *de novo* NAD^+ biosynthesis, and requires exogenous NAD^+ precursors to grow. In the absence of exogenous NAD^+ precursors, *C. glabrata* stops growing but remains viable for up to seven days. Strikingly, NAD^+-starved cells are highly virulent relative to glucose starved cells. We present our current understanding of how NAD^+ cellular status affects the pathogenesis of *C. glabrata*.

A key feature important to the virulence of *C. glabrata* is its array of cell wall proteins encoding genes. *C. glabrata* encodes approximately 80 adhesin-like cell wall proteins, including a large family of lectins that mediate binding to mammalian cells. Our resequencing of the *C. glabrata* genome has clarified aspects of genome organization and defined the structure and complement of adhesin-like proteins in this organism. Many of these adhesin-like proteins are encoded within the sub-telomeric regions of the chromosome, where they are transcriptionally repressed by Sir2-mediated chromatin modification. Since sirtuins require NAD^+ as an enzymatic cofactor, we have examined how NAD^+ cellular levels alter transcription of the sub-telomeric adhesins and the impact on virulence.

In addition to the impact of NAD^+ on sirtuin function and gene regulation, we have characterized a broad response to NAD^+ depletion that is independent of the sirtuins. We show unexpected connections between NAD^+ cellular levels and *de novo* purine metabolism. We will present metabolomic and genetic studies connecting NAD^+ and cellular purine levels and exploring their impact on the virulence of *C. glabrata*.

Mitochondrial protein biogenesis: A huge challenge for eukaryotic cells

Johannes M. Herrmann

Cell Biology, University of Kaiserslautern, Erwin-Schrödinger-Strasse 13, 67663 Kaiserslautern, Germany

Most mitochondrial proteins are synthesized as cytosolic precursor proteins before being imported into mitochondria. The early reactions in the targeting of mitochondrial precursor proteins are largely unclear. We employed a genetic high-throughput screen to identify factors critical for the intracellular sorting of the mitochondrial membrane protein Oxa1. Unexpectedly, we found several components of the ER membrane that are critical for the intracellular transport of Oxa1. By use of a combination of biochemical and genetic approaches, we identified a novel intracellular targeting route for mitochondrial proteins, which directs them to the ER surface where they are maintained in an import-competent conformation. From there, they are handed over to the mitochondrial TOM complex by use of dedicated components, such as the ER-bound J protein Djp1.
In order to study the response triggered by the cytosolic accumulation of precursor proteins, we employed “clogger” proteins that compete with other precursors. Deep-sequencing of cellular mRNAs upon induction of the “clogger” proteins revealed a global transcriptional program to restore cellular proteostasis. This transcriptional remodeling is a combination of a “wideband” core response that is similar to the general heat shock response and a unique mitoprotein-induced downregulation of the oxidative phosphorylation components. These findings reveal the first comprehensive, time-resolved model of adaptations to mitochondrial import impairment.
In summary, in my talk I will report about reactions of mitochondrial precursor proteins that occur outside of mitochondria which document novel, so far largely unexplored aspects of cellular biology.

From sex to apomixis: evolution, control, and induction of clonal reproduction.

Jean-Philippe Vielle-Calzada,
Grupo de Desarrollo Reproductivo y Apomixis. UGA Langebio Cinvestav Irapuato, México.

Perhaps owing to Darwin´s failure to shed light in the central explanation of why sex is so prevalent in all branches of life, several decades of theoretical studies have yet to provide a reasonable explanation for the evolutionary emergence of mechanisms that can give rise to a viable embryo through asexual methods of clonal reproduction. Apomixis refers to a set of reproductive mechanisms that invariably rely on avoiding meiotically derived gamete reduction and fertilization of the oocyte to generate clonal seeds in flowering plants. After being long considered a strictly asexual oddity leading to extinction, the integration of more than 100 years of embryological, genetic, molecular and ecological research have revealed its importance as a widely spread component of the dynamic processes that shape plant evolution through several flexible and versatile developmental pathways. I will review our current findings related to the mechanisms controlling unreduced gamete formation, haploid induction, and parthenogenesis, emphasizing similarities and differences between sex and apomixis, and highlighting their implications for the evolutionary emergence and induction of asexual reproduction through seeds. On the basis of these comparisons, I will propose a model that associates the developmental origin of apomixis to a dynamic epigenetic landscape in which environmental fluctuations reversibly influence female reproductive development through mechanisms of hybridization and polyploidization.

Communication of cellular misfolding across tissues for enhanced longevity

Andrew Dillin
University of California, Berkeley, USA

Rotavirus strategies to fight-back the antiviral responses of its host-cell.

Susana López Charretón
Instituto de Biotecnología/UNAM

Abstract:

General stress responses and innate immune responses are intimately linked and interface at many levels. The outcomes of these responses serve to reprogram host gene expression patterns to prevent viral invasions. In turn, viruses counter-attack these cell responses to ensure their replication. The mechanisms through which viruses attempt to control host cell responses are as varied as the number of different virus families. Interestingly, one of the initial steps to control the antiviral response of the cell, and a very resorted solution used by several virus families is to hijack the translation machinery of the host, such that the translation of viral proteins is ensured, while the expression of the stress and antiviral responses of the cell are blocked at the translation level.

Rotaviruses are one of the most important causes of acute gastroenteritis in childhood, causing about 220,000 deaths per year in the world in children under two years of age.

As in any other viral infections, rotaviruses trigger an antiviral response in their host cell. We are interested in learning how these viruses deal with the different branches of this response that are turned on upon infection. We have found that early on infection rotavirus induces a shut-off of the cell protein synthesis in which several cellular components of the translation machinery are compromised by the virus. During infection the accumulation of RNA granules is also altered, and the nucleo-cytoplasmic localization of several RNA binding proteins, which are important constituents of RNA granules, is redistributed. Additionally, we have also found that the OAS-RNAse L system, which is one of the initial antiviral measures of the cell upon sensing dsRNA, becomes disabled during rotavirus infection. In this seminar, I will present our recent advances in these topics.

Recambio de las “Señoritas Neuronas": mecanismos y limitaciones.
Neuronal replacement in the adult brain: mechanisms and limitations.

Arturo Álvarez-Buylla Roces
Department of Neurological Surgery and
The Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research
University of California, San Francisco, School of Medicine
San Francisco, CA 94143-0525

The demonstration that neuronal birth, migration and differentiation continue postnatally raises questions about its mechanisms and suggests new approaches for brain repair.

Previous work in the laboratory identified a large population of neural stem cells (B1 cells) in the walls of the lateral ventricles of the adult rodent brain. B1 cells generate intermediate progenitors (C cells) that in turn give rise to large numbers of young neurons that migrate a long distance to the olfactory bulb through the rostral migratory stream. In the olfactory bulb, the newly formed neurons differentiate into 10 different types of local-circuit inhibitory cells that continually replace older neurons that die. In the neurogenic niche of the ventricular-subventricular zone (SVZ), where these neurons are born, B1 cells share the epithelial compartment with ependymal cells (E1 cells). B1 cells’ small apical ending is surrounded by the large apical surfaces of the multiciliated ependymal cells (E) forming striking, flower-like arrangements denominated pinwheels.

I will address three questions during the first part of my lecture: 1) what is the origin of B1 and E1 cells during development, 2) how do the pinwheels form, and 3) what is the mechanism by which B1 cells generate the different types of neurons. Recent findings have shown that after their generation in the embryo, B1 cells remain quiescent until re-activated to produce neurons in postnatal life. Furthermore, in contrast to current views, we find no evidence for the asymmetric self-renewal of B1 cells. This raises a fourth question: 4) how B1 cells maintain neurogenesis for life. Opportunities for brain repair will be discussed, in the context of both answers to the above and new observations on the distribution of young neurons in postnatal human brain.

ABC Transporters in Nutrient Homeostasis and Pathogenesis

Heather W. Pinkett

Department of Molecular Biosciences
Northwestern University, Evanston IL 60208

Proteins located in the cell membrane work as gatekeepers to selectively allow compounds into or out of the cell. Such gatekeepers are known as or ATP-binding cassette (ABC) transporters, because they use the energy of ATP (adenosine triphosphate) hydrolysis to transport compounds across the cell membrane. We are interested in (1) *How ABC transporters use specific and sometimes interchangeable components of the transport system to transport substrates?* (2) *How has the mechanism of substrate selectivity evolved to allow for promiscuous substrate transport?* Bacterial ABC importers are essential for organism survival, controlling the rate of uptake for nutrients scavenged from the bacterium's environment. Control of the rate of transport precludes over-accumulation of a nutrient that is beneficial at low concentrations, but is potentially toxic at high concentrations. Our results provided new insights into organisms that use multiple transport systems to regulate nutrient influx of a range of substrates. Defining the molecular mechanism that controls nutrient uptake also allows us to understand how multicomponent transport systems work in concert to recognize and circumvent the host innate immune response, a mechanism crucial to the evolution of antimicrobial peptide (AMP) resistance in pathogenic bacteria.

From genomics to levitating cells and rare diseases

Lars M. Steinmetz
EMBL Heidelberg/Stanford Genome Technology Center. Meyerhofstraße 1, 69117 Heidelberg, Germany

Medicine is undergoing a transformation from a one-size-fits-all, reactive system to one that is predictive, preventive, personalized and participatory. My lab develops and applies novel technologies to facilitate that transformation. We have modelled rare genetic diseases using genome editing in induced pluripotent stem cells and thereby identified, among other things, a potential pharmacological strategy for preventing dilated cardiomyopathy in genetically predisposed individuals. We have developed magnetic levitation as a low-cost, high-throughput solution for separating circulating tumour cells (CTCs) from blood, a strategy we believe will transform CTC characterisation.

Much of the progress towards personalized medicine is based on technologies to read, edit and write genomes across scales. Genomes can now be read at the resolution of single cells. We have used single cell transcriptomics to redefine how human blood is made, to study promiscuous gene expression in the thymus and to chart the spatial organisation of adult stem cell niches. Genomes can now be edited precisely, genome-wide. Using CRISPR-Cas9 we have introduced 10,000s of single nucleotide variants one at a time in yeast, allowing us to measure the effects of single nucleotide perturbations in isolation. Finally, entire genomes can now be written from scratch. With designer chromosomes and a yeast strain composed entirely of synthetic DNA we are studying how genome organization shapes the transcriptional landscape and how abrupt changes to this organization influence cellular evolution.

Together, these genetic technologies are fundamentally reforming the types of questions that can be addressed with experimental approaches, which will in turn have profound implications for medicine, industrial biotechnology and our understanding of life’s basic organizing principles.

Therapeutic tRNA synthetase Inhibition Activates a Novel Arm of the Amino Acid Response Pathway.

Whitman M[1], Keller T[1], Sundrud M [2], Yeo CY[2] Kim YJ[1], Zocco, D. [4]Rao, A., Edenius,M., Zhou CQ[1]
1Department of Developmental Biology, Harvard School of Dental Medicine, Department of Cell Biology, Harvard Medical School.
2 Scripps Research Institute, Jupiter FL
3Ewha Women's University, Seoul Korea
4 La Jolla Institute for Allergy and Immunology, La Jolla CA
E-mail: mwhitman@hms.harvard.edu

Aminoacyl-tRNA synthetases (AARSs) play an essential role in cellular protein translation, a property that has led to widespread use of pathogen-directed AARS-inhibitors as antimicrobials. Our lab recently identified a novel therapeutic effect of AARS-inhibition by showing that low-dose halofuginone (HF)-treatment activates the mammalian Amino Acid Response (AAR) pathway, thereby suppressing tissue inflammation through hormetic prolyl-tRNA synthetase-inhibition. We now show that low-dose HF-treatment confers therapeutic benefit through the selective inhibition of inflammatory responses in a variety of cell types, that the threonyl-tRNA synthetase-inhibitor borrelidin elicits many of these same responses, and that the therapeutic effects of HF and borrelidin are seen in cells lacking the canonical AAR pathway-effector GCN2. These findings establish that the generation of uncharged tRNAs through tRNA synthetase inhibition have broad therapeutic effects for chronic inflammatory disease through a pathway independent of the major tRNA sensor GCN2.

Mycobiota of Greenland Ice Sheet for a more sustainable world

Nina Gunde-Cimerman

Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, SI-1000 Ljubljana, Slovenia, EU

Water is crucial for life as we know it. Freezing leads to decreased water activity, invasion of toxic ions of inorganic salts into the cells, formation of ice crystals inside and outside the cells, all leading to disturbance of biological systems. Psychrophilic/psychrotolerant fungi have evolved specialized molecular mechanisms for avoiding and managing these detrimental effects. Since polar environments promote competition for the scarce resources, these fungi often synthesize novel and unique antibacterial, antifungal and/or antialgal compounds and enzymes functional at low temperatures and high salinity. These characteristics make them interesting for biotechnological applications, such as biocontrol or degradation of natural or artificial pollutants in cold and /or Arctic areas.
Only recently mycobiota of Greenland ice sheet, in particular associated to non-cultivable black ice algae, has been discovered. Environmental data and information on the fungal biodiversity, different life strategies observed so far and mechanisms of adaptations will be presented.

Key words: glaciers, ice-sheet, low water activity, *Articulospora sp.*, *Penicillium bialowiezense*-like, ice algae.

The components of the fungal cell wall change the polarization state of human macrophages

Emmanuel Orta Zavalza, Brenda Lizbeth Estrada Capetillo and Angel Luis Corbi Lopez.

Centro de Investigaciones Biologicas (CIB), Consejo Superior de Investigaciones Cientificas (CSIC), Madrid, Spain
Universidad Autonoma de Ciudad Juarez, Departamento de Ciencias Quimico-Biologicas, Av. Benjamin Franklin 4650, Zona PRONAF, CP. 32310, Juarez, Chihuahua, Mexico.
Phone: +52 (444) 369-5981 email: emmanuel.orta@uacj.mx

Disseminated candidiasis is an important public health problem because it is associated with high mortality rates among immunocompromised patients. *Candida albicans* prevails as the main causative agent of candidiasis and uses different strategies to evade the innate immune response, such as surviving within the phagocytes. Along with neutrophils, macrophages are phagocytic cells that neutralize fungal pathogens during infection. Macrophages are cells with a high functional plasticity that in the presence of pathogens acquire pro-inflammatory properties (M1). In contrast, other stimuli lead macrophages to an anti-inflammatory state (M2) which is associated with tissue repair and pro-angiogenic activities. It has been proposed that some microorganisms redirect the profile of macrophages towards an anti-inflammatory phenotype to evade the immune response. Some evidences suggest that *C. albicans* alters the polarization state of pro-inflammatory macrophages (M1) towards an anti-inflammatory phenotype (M2) and this could explain the persistence of the fungus in recurrent infecitons. To gain insight into this phenotypic change we have analyzed the cytokine production and the modifications in the signaling pathways of human polarized macrophages in response to different components of the cell wall of *C. albicans*. So far, our results indicate that the morphologic state of the fungus influences the magnitude of the response in both population of macrophages. We observed a fast phosphorylation of the ERK1/2 pathway and a greater production of cytokines when macrophages were stimulated with β-glucan derived from hyphae whereas this change was not observed with the yeast glucan. Our findings also demonstrate that hyphal β-glucans induce the production of IL-10, a potent anti-inflammatory cytokine, in M2 macrophages. In contrast, mannans did not alter the production of cytokines in any of the macrophage types. This finding supports the masking model which suggests that mannan acts as a shield that protects β-glucan from being recognized by the cells of the host. How *Candida* species alter the behavior of macrophages during infection is currently investigated by our research group.

MYO-5 the single actin-associated molecular motor in *Neurospora crassa*

Arianne Ramírez-del Villar[1], Robert W. Roberson[2], Olga A. Callejas-Negrete[1], **Rosa R. Mouriño-Pérez[1]**

[1]Departamento de Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada. Ensenada, Baja California, Mexico.
[2]School of Life Sciences, Arizona State University, Tempe, Arizona, USA

In filamentous fungi, polarized growth is the result of vesicles secretion at the hyphal apex. Motor proteins mediate vesicle transport to target destinations on the plasma membrane via actin and microtubule cytoskeletons. Myosins are motor proteins associated with actin filaments. Specifically, class V myosins are responsible for cargo transport in all eukaryotes. We studied the dynamics and localization of myosin V in wild type hypha of *Neursopora crassa* and in hyphae that lacked MYO-5. In wild type hyphae, MYO-5-GFP is localized in the hyphal apex and colocalized with Spitzenköper. Photobleaching studies showed that MYO-5-GFP was transported to the apex from subapical hyphal regions. The absence of the class V myosin resulted in reduced rates of hyphal growth, apical hyperbranching, and intermittent loss of hyphal polarity. MYO-5 myosin did not participate in breaking the symmetrical growth during germination (referring to swelling of conidia?) but contributed in the apical organization upon establishment of polarized growth. In the Δmyo-5 mutant, actin was organized into thick cables in the apical and subapical hyphal regions, and the number of endocytic patches were reduced. CHS-1-GFP was distributed as a cloud occupying the apical dome and not in the Spitzenkörper as the WT strain. The mitochondrial movement was not associated with MYO-5, but tubular vacuoles position is dependent. These results suggest that MYO-5 plays a role in maintaining apical organization and a robust Spitzenkörper and is required for radial growth, hyphal polarity, septation, conidiation, and proper conidial germination.

IRES-dependent translated genes in fungi: Computational prediction, phylogenetic conservation and functional association.

Enrique Merino, Liliana Pardo and Esteban Peguero-Sanchez

Instituto de Biotecnología – UNAM. Av. Universidad 2001, Cuernavaca, Morelos.
CP 62210, Mexico. Tel: 7773114904. Email: merino@ibt.unam.mx

The initiation of translation via cellular internal ribosome entry sites plays an important role in the stress response and certain physiological conditions in which canonical cap-dependent translation initiation is compromised. Currently, only a limited number of these regulatory elements have been experimentally identified. Notably, cellular internal ribosome entry sites lack conservation of both the primary sequence and mRNA secondary structure, rendering their identification difficult.Despite their biological importance, the currently available computational strategies to predict them have had limited success. We developed a bioinformatic method based on a support vector machine for the prediction of internal ribosome entry sites in fungi using the 5'-UTR sequences of 20 non-redundant fungal organisms.

Additionally, we performed a comparative analysis and characterization of the functional relationships among the genetic products that are predicted to be translated by this cap-independent mechanism, as it could be evaluated by the statistical analysis of the enriched Gene Ontology (GO) terms. It is worth noting that a number of these enriched GO terms have been associated with protein synthesis through 5'-cap-independent translation in a selective manner in growth conditions including developmental processes, transport, cell communication, filamentous growth and response to stress.

Furthermore, in order to have an indirect proof of the biological significance of our predictions, we search in the literature for genes in other organisms that have experimental evidence of being translated by IRES. We found statistically significant conservation of IRES-dependent translation in some groups of orthologous genes that revealed an underlying selective pressure, particularly in stress related genes. This is the case of the HSP70 chaperone family, which remarkably IRES has been verified in humans and flies. A second example is the orthologous group of the eIF4G repression protein Sbp1p, which has two homologous genes known to be translated by this cap-independent mechanism, one in mice and the other in yeast. These examples emphasize the wide conservation of these regulatory elements as a result of selective pressure. In addition, we performed a protein-protein interaction network characterization of the gene products of our positive predictions using *Saccharomyces cerevisiae* as a model, which revealed a highly connected and modular topology, suggesting a functional association. A remarkable example of this functional association is our prediction of internal ribosome entry sites elements in three components of the RNA polymerase II mediator complex.

In conclusion, our study represents a useful resource for hypothesis-driven experiments and gene function exploration in the field of cap-independent translational regulation.

Conjugative transfer of rhizobial plasmids in plant nodules

Susana Brom[(1)], Luis Alfredo Bañuelos-Vázquez[(1)], Gonzalo Torres Tejerizo[(2)], Lourdes Girard[(3)], Laura Cervantes de la Luz[(1)] and David Romero[(1)].

Programas de Ingeniería Genómica[(1)] and Biología de Sistemas y Sintética[(3),] Centro de Ciecias Genómicas, UNAM. Av. Universidad 1001, col. Chamilpa, Cuernavaca, Mor., México. sbrom@ccg.unam.mx. IBBM CCT-CONICET-La Plata, Universidad Nacional de La Plata. La Plata, Argentina[(2).]

Rhizobium etli strain CFN42 is able to fix nitrogen in symbiosis with the roots of bean plants. Establishment of the symbiosis starts with a chemical communication involving plant exudates and bacterial compounds. This causes an invagination of hair root cells to form an *infection thread* used by the bacteria to penetrate the root and invade plant cell derived *nodules*, where the bacteria are able to fix atmospheric nitrogen, converting it to organic forms which are provided to the plant cells in exchange for carbon sources. *R. etli* CFN42 has one chromosome and 6 large plasmids (pRet42a to pRet42f). Genes required for the symbiosis and other functional genes are carried by the plasmids. We have previously shown that plasmid pRet42a is able to perform conjugative transfer (CT). Expression of the CT related genes is regulated by quorum-sensing through a *traI* encoded homoserine-lactone and transcriptional regulators encoded by *traR* and *cinR*.

In this work, we explored the capacity of pRet42a to perform CT on the surface of roots, and inside the symbiotic structures. To do this we used a donor labelled on the chromosome with a RFP marker and a GFP marker on pRet42a and an unlabelled recipient. So, when CT takes place we can differentiate donors (yellow), recipients (no color) and transconjugants (green) (Torres Tejerizo *et al.,* 2015. J. Microbiol. Methods. Vol 117:155-163). The analyses were done by fluorescence microscopy, cytometry and CFU. The results showed transconjugants on the root surface, in the infection threads and inside the nodules. The presence of transconjugants in the nodules could be due to CT taking place on the root surface and the transconjugants establishing symbiosis, and/or, CT might also be occurring inside the nodules. To discern between these alternatives, we analysed the: 1) expression status of transfer related genes in nodules and infection threads, 2) generation of transconjugants inside nodules when CT was partially inhibited on the root surface using the *traM* gene, which is an anti-activator of TraR, 3) presence of transconjugants inside nodules when CT is completely inhibited on the root surface, by placing the *traI* gene under control of the nitrogenase (*nifH*) gene promoter, which is only activated inside nodules, and 4) decrease in tranconjugants in the nodules when the *traM* antiactivator is higly induced in this structure, by placing the gene under the *nifH* promoter.

Our results show that pRet42a can be transferred inside nodules, and not only on the root surface. This finding broadens the view of nodules, as structures where bacteria may exchange genetic material and diversify, representing a clear benefit for the bacteria.

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Identification of small RNAs and small peptides in *E. coli.*

Juan Miranda Ríos[1] and Gisela Storz[2].

[1]Unidad de Genética de la Nutrición. Instituto de Investigaciones Biomédicas UNAM and Instituto Nacional de Pediatría.Torre de Investigación. Av del IMAN #1, 4º Piso. Coyoacán, Ciudad de México 04530.Tel: 55-5622 6423.
E-mail:riosjuanm@biomedicas.unam.mx.
[2]National Institute of Child Health and Human Development, NIH, USA.

Bacteria were thought to contain only a few categories of **RNAs**: tRNAs, rRNAs, and mRNAs, plus a few specialized RNAs found in RNase P, the signal recognition particle, and the ribosome rescue system. In this context, gene expression was seen to be controlled almost exclusively at the level of transcription, in which protein levels were linearly related to mRNA levels. Over the past 20 years, we have witnessed a revolutionary growth in diversity, mechanisms and functions of RNA molecules in bacteria. Some are involved in the response to different cellular and environmental stimulus to adjust mRNA expression and are present in the 5´ UTR of their mRNAs, such as the metabolite-sensing riboswitches and the RNA thermometers. Other, called small non-coding RNAs (**sRNAs**), modulate mRNAs by diverse mechanisms, often in association with conserved RNA binding proteins. Another class contains the sRNAs from 3´mRNA regions and the antisense RNAs. It is predicted that *E. coli* and *Salmonella* contain approximately 300 **sRNAs**, a number comparable to that of transcription factors, and that at least half of all mRNAs are subject to sRNA-mediated regulation. These **sRNAs** and their target mRNAs constitute a large and complex regulatory network in bacteria. Other molecules that had been overlooked are the small open reading frames (**sORFs**) that code for small proteins of less than 50 amino acids.

The *E. coli* chromosome encodes more than 60 **sORFs**, but their functions remain mostly unknown. However, as well as for **sRNAs**, many are conserved and/or are produced under precise environmental conditions, implying that they entail critical physiological functions.

Previously, computational and experimental searches allowed the identification of 60 **sRNA** genes in *E. coli*. By means of a cloning-based screen of RNAs 30-65 nt, we were able to identify 20 additional **sRNAs** that corresponded to 5´- and 3´-untranslated regions (UTRs), internal fragments of mRNAs, three additional sRNAs in intergenic regions, antisense RNAs that putatively base-pair with the 5´or 3´ ends of their target mRNAs.

Additionally, we assayed the accumulation of 60 *E. coli* **sORFs** under a variety of growth conditions and after exposure to stress. For some genes, the observed changes in protein levels were compatible with known transcriptional regulation by ArcA, Zur, or cyclic AMP response protein (CRP), showing that **sORFs** have been an overlooked set of stress response proteins in *E. coli* in particular, and bacteria and eukaryotes in general.

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Life in a microbial community: get by with a little help from your friends

Gabriela Olmedo Álvarez

Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Unidad Irapuato
Km. 9.6 libramiento Norte Carretera Irapuato-León Cp. 36825, tel. 014626239602,
golmedo@cinvestav.mx

One of my group's interest is to understand the interactions that take place among members of sediment communities, in particular, those in the Churince water system, in Cuatro Cienegas, Coahuila. How is the amazing diversity maintained in a place with extreme phosphorus limitation? How can we possibly understand the interactions among hundreds of species? It is believed that bacteria that are genetically closer share a similar metabolism and thus can be stronger competitors than species genetically distant. We therefore focused our studies on a single genus, the *Bacillus*, to look deep at the genetic and phenotypic diversity, and have implemented synthetic ecology strategies to study their interactions. We have previously shown that antagonistic interactions influence communities structre.

Networks of antagonistic interactions between different *Bacillus* species allowed us to model these interactions using *in silico* "cell automaton" strategies. In this talk, I'll

present our results on synthetic communities and the emergent properties observed. I'll also show you data based on mesocosm studies aiming at obtaining information about the influence of a complex community environment on particular bacterial species. From Petri dish studies to transcriptome analysis, we have been able to explore ecological interactions that help us to define principles that underly the stability and performance of microbial communities. One of the principles uncovered is that diversity can transform an antagonistic community into one that shields sensitive bacteria from antagonism. A better understanding of microbial competition will allow us to better predict the behaviour of microbes, to control and manipulate microbial communities for industrial, environmental and medical purposes.

Distinctive microbiome in the southwestern Gulf of Mexico, a historically impacted zone

Tina Godoy*, Alejandra Escobar,* Luciana Raggi *, Adolfo Gracia°, Rosa María Gutierrez*, Katy Juárez*, Enrique Merino*, Lorenzo Segovia*, Alexei Licea§, Alejandro Sánchez*, **Liliana Pardo-López*.**

*Instituto de Biotecnología, UNAM
°Instituto de Ciencias del Mar y Limnología, UNAM
§Centro de Investigación Científica y de Educación Superior de Ensenada.

The Gulf of Mexico is recognized worldwide as an ocean basin with large oil resources that benefits the economies of the surrounding countries. At the same time it is considered a stressed ecosystem by the presence of natural leaks and oil spills. Studies from marine sediments in the North of the Gulf of Mexico reveal important microbial communities that are enriched with hydrocarbon-degrading bacteria at sites affected by oil spills, however, there are no studies in the southwest where there are rich oil zones. To characterize the basal state of microbial diversity from the southwest of Gulf of Mexico, represent a unique opportunity to study the indigenous populations of bacteria and its hydrocarbon degrading potential. In this work, we described for the first time the bacterial and geochemical landscape for the swGoM establishing the first taxonomic pan genera for marine sediments in this region. Interestingly, we found a strong correlation of already reported bacterial genera capable of aromatic hydrocarbon degradation, and some new organism with hydrocarbon degrading potential, in samples that have been chronically exposed to PAHs in shallow sediments. The unique presence of several core genera in the swGoM was confirmed after a comprehensive comparative metagenomics approach, against results from five different marine sediment sequencing projects. To our knowledge, this is the first study in the swGoM that provides clues to the bacterial population acclimation, to the ubiquitous presence of hydrocarbons that shaped and selected organisms with biotechnological and ecological surveillance applications.

Metabolic modeling and control analysis of *Trypanosoma cruzi* antioxidant defense

Emma Saavedra, Citlali Vázquez, Zabdi González-Chávez

Department of Biochemistry. Instituto Nacional de Cardiología. 14090, Mexico City. MEXICO. emma_saavedra@hotmail.com

In the search for therapeutic targets in pathogenic cells, the gene essentially criterion determined by using knock-out or knock–down genetic strategies is commonly applied. However, it is often found that most or all of the enzymes/proteins analyzed are indeed essential; therefore, additional criteria have to be implemented for drug target identification and validation. In this regard, kinetic metabolic modeling (a bottom-up Systems Biology approach) and the fundamentals of Metabolic Control Analysis allow identification of the fluxcontrolling steps in metabolic pathways and helps in the understanding of the mechanisms of control and regulation of metabolism. These two approaches were applied to the antioxidant metabolism of the parasite *Trypanosoma cruzi*, the causal agent of human Chagas disease to validate drug targets. Trypanothione (T(SH)2) is the main antioxidant metabolite for peroxide reduction in the parasite and replaces the antioxidant function that glutathione has in human cells.

Syntheses of T(SH)2 and its precursor GSH are catalyzed by gamma-glutamil cysteine synthetase (γECS), glutathione synthetase and trypanothione synthetase (TryS). Further, the hydroperoxide reduction pathway transfers the reductive equivalents from T(SH)2 to tryparedoxin (TXN) and then to tryparedoxin peroxidase (TXNPx); finally, oxidized trypanothione is reduced by trypanothione reductase (TryR). To determine the degree of control that each enzyme has on the pathway flux (flux control coefficient *CJ ai*) the expression of γECS, TryS, TryR and TXN was manipulated in the parasites and the *CJ*

ai were determined as well as the role of these enzymes on parasite´s antichagasic and peroxide resistance, differentiation and infectivity. Moreover, computational kinetic models of the two pathways were constructed which were able to closely simulate the pathway behavior in the parasites. *In vivo* and *in silico* analyses indicated that γECS and TryS control T(SH)2 synthesis by 62-75% and 15-27%, respectively. γECS overexpression prompted up to 4-fold increase in T(SH)2 concentration. The peroxide reduction flux was controlled 72-76% by TXN, 16% by TXNPx and 12-15% by TryR. TXN and TryR overexpression increased H2O2 resistance whereas γECS, TryS and TXN increased resistance to benznidazole *plus* buthionine sulfoximine. Only γECS overexpression led to an increase in infectivity capacity. The data suggested that γECS and TXN inhibition should strongly compromise the parasite viability and infectivity. In contrast, since TryR, the preferred target for drug design studies, exhibited low flux-control, its use as drug target is unsuitable because it would be required highly potent and specific inhibitors to decrease the antioxidant defense in the parasite. The results also indicated that high thiol metabolites and small dithiol proteins provide drug resistance to antichagasic drugs. This investigation demonstrates that controlling enzymes/transporters can indeed be proposed as the most convenient therapeutic targets to interfere in parasite metabolism.

Modulating the redox state: an alternative to modify the behavior of senescent cells in the aging brain

Mina Königsberg.
Departamento de Ciencias de la Salud, División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana-Iztapalapa, A.P. 55-535. C.P 09340, Mexico City, Mexico. Phone: (5255) 5804-4728; Fax: (5255) 5804-4727; E-mail address: mkf@xanum.uam.mx

Cellular senescence is a process that occurs as a response to diverse stressors and has been proposed as an important hallmark of aging. Senescence is characterized by the loss of proliferative capacity, DNA damage, an increase in β-galactosidase activity and an inflammatory secretion profile. Senescent cells release a complex set of cytokines, chemokines and growth factors that are collectively known as SASP (Senescence-Associated Secretory Phenotype). The SASP is known to induce both beneficial and detrimental effects on adjacent cells, as tissue remodeling, would healing, as well as tumor suppression and promotion. The SASP components vary depending on the cell type, senescence induction and time, but their main regulatory pathways are determined by the cellular redox-state. The SASP has been studied in fibroblasts and epithelial cells, but little is known in the context of the Central Nervous System.

Brain aging is a complex process that is characterized by morphological and neurochemical alterations, and recently senescent cells have been shown to play a role in neurodegeneration. So, one of the most important challenges in aging biology is to understand the mechanisms involved in this progression, in order to relate the biochemical alterations to the functional decline during old age. Here, we analyzed if the SASP profile of senescent astrocytes induced to senescence by oxidative stress (SIPS) or by proteasome inhibition (PIIPS), was modified after being treated with two drugs that are known to modify redox state: Sulforaphane (SFN) and Dehydroepiandrosterone (DHEA).

In order to evaluate the effect of the SASP secreted by the senescent astrocytes on the neuronal function, cellular co-cultures in transwell plates were performed and the mitochondrial membrane potential and synaptic potential in the neurons were evaluated.

These parameters were chosen because those characteristics are lost during neurodegeneration.

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Molecular Regulation of Renal Salt and Potassium Metabolism

Gerardo Gamba, MD, PhD

The renal thiazide-sensitive NaCl cotransporter (NCC) is the major salt transport pathway in the distal convoluted tubule of the mammalian nephron. NCC activity is critical for modulation of arterial blood pressure and serum potassium levels. The activity of NCC constitutes a switch that modulates potassium excretion. Decreased NCC activity is associated with salt and potassium excretion while increased activity with salt and potassium retention. Thus, reduced activity of NCC in genetic diseases results in arterial hypotension and hypokalemia, while increased activity results in genetic diseases featuring hypertension and hyperkalemia. Several hormones and physiological conditions modulate NCC activity through a final intracellular complex pathway involving kinases (With-no-lysine kinases, WNKs) and ubiquitin ligases (KLHL3 and CUL3). A substantial amount of work has been conducted to understand this pathway in the last 15 years, but advances over the last three years have helped to begin to understand how these regulatory proteins interact with each other and modulate the activity of this important cotransporter. In this talk I will present the current model of NCC regulation by the CUL3/KELCH3-WNK-SPAK pathway and will discuss the genetic diseases in humans affecting this pathway as well as all the genetically altered mice that have been used to translate most of the proposals made from *in vitro* experiments into *in vivo* observations that have helped to elucidate the model at the physiological level. Many questions have been resolved, but some others will require further models to be constructed. In addition, unexpected observations in mice have raised new questions and identified regulatory pathways that were previously unknown.

Molecular Mechanisms of Insulin Resistance induced by Fatty Acids

Jesus Alberto Olivares-Reyes*, Adriana Roura-Guiberna, J. Gustavo Vazquez-Jimenez and Judith-Hernandez Aranda
Department of Biochemistry, CINVESTAV-IPN, Av. IPN 2508, Col. San Pedro Zacatenco, Mexico City, CP 07360 Mexico. E-mail: jolivare@cinvestav.mx, Phone 5255-5747-3951.

Free fatty acids (FFA) are essential nutritional components in mammalians, and they act as an important source of energy for most tissues of the body, including heart, skeletal muscle, and liver. Also, they possess a wide range of biological functions, such as an essential component of the cell membrane (being crucial to its fluidity and functionality) and acting as signaling molecules in various physiological processes. It has now been shown that increased plasma concentrations of FFA in humans are associated with insulin-resistance states in obese patients with type 2 diabetes mellitus (DM2). Typically, saturated fatty acids (SFA), such as palmitic acid (PA), have been found to play a critical role in the development of insulin resistance. For example, in human umbilical vein endothelial cells (HUVEC), we showed that PA induces insulin resistance through a mechanism that involves inhibition of SERCA pump activity that leads to endoplasmic reticulum stress and the consequent activation of JNK. After its activation, JNK can block the insulin-signaling pathway resulting in a state of insulin resistance. Additionally, PA also induces Akt inactivation in hepatic C9 cells, promoting not only insulin resistance but also lipid accumulation in this cell model. Interestingly, and contrary to what has been documented with SFA, polyunsaturated fatty acids (PUFA) represent a group of FFA with anti-inflammatory and anti-diabetic effects. Conjugated linoleic acids (CLA) are a family of PUFA, with several beneficial effects on human health, particularly in inflammation, hypertension, diabetes, and insulin resistance, among others. However, recent reports have suggested that these observed effects on human health are isomer-specific. In a recent study from our laboratory, we found that in hepatic C9 cells, c9,t11-CLA- and t10,c12-CLA-isomers, two of the main isomers of CLA associated to their biological actions, inhibit insulin-induced Akt/GSK3/GS signaling pathway through phosphorylation of IRS-1 at Ser612 and Ser307, by a mechanism that involves activation of PKC□. Additionally, we found that CLA-isomers induce lipid droplets accumulation and modify the morphology of hepatic cells. In contrast with these observations, linoleic acid (LA), which has been associated to protect against obesity-induced insulin resistance and improve glucose tolerance, induced an evident sensitization effect on insulin-induced signaling in hepatic C9 cells. In summary, our findings with CLA-isomers are associated with decreased insulin signaling promoting the inability of insulin to trigger glycogen synthesis in hepatic cells and the development of liver damage, including hepatic steatosis. Thus, our results also suggest the caution that must be taken when considering the therapeutic potential of CLA and others FFA in insulin resistance.

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An alternative polyamine pathway mediates lysine plasticity in response to H_2O_2 stress in *Saccharomyces cerevisiae*

Viridiana Olin Sandoval[1,2], Leonor Miller-Fleming[2], Robert Haas[2], Markus Ralser[2]
[1]Department of Food Science and Technology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán.14090 Mexico City, Mexico. Tel. 54870900 Ext. 1575. violin1226@gmail.com
[2]Department of Biochemistry, University of Cambridge. CB2 1GA, Cambridge UK.

Polyamines (PAs) are essential metabolites associated to various aspects of stress tolerance1, however the underlying molecular mechanisms are not completely understood. Previously, it was determined that in *Saccharomyces cerevisiae* the polyamine export mediated by Tpo1 was involved in the antioxidant protection by controlling the spermine and spermidine intracellular concentrations; and inducing the expression of stress related proteins[2]. Moreover, the proteomic analysis of the H_2O_2 stress response in WT and Δ*tpo1* strains, revealed a connection between lysine metabolism and the PA export. Searching for this link we found that, upon incubations with H_2O_2, yeast cells uptake more lysine and decarboxylate it to form the PA cadaverine. The increase in lysine consumption inhibited its biosynthesis pathway, promoting a re-routing of NADPH that was reflected on an increment in reduced glutathione levels, a decrease of ROS concentrations and thus in a higher H_2O_2 tolerance.
Besides, we identified that cadaverine was synthesized from lysine by an alternative reaction of Ornithine Decarboxylase that, even though has a low affinity for this amino acid, it was able to decarboxylate it when the cells were supplemented with lysine. The function of this alternative polyamine pathway in the stress response in yeast still needs to be elucidated. Thus, until now, this pathway seems to be an unintended side-effect of the anti-stress mechanism involving lysine.

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On the conformational stability of natural and *de novo* TIM barrels.

Daniel Alejandro Fernández Velasco[1], Sergio Romero Romero[1], Ricardo Muñíz Trejo[1], Adela Rodríguez Romero[2], Miguel Costas[3].
[1]Laboratorio de Fisicoquímica e Ingeniería de Proteínas, Departamento de Bioquímica, Facultad de Medicina. [2]Departamento de Química de Biomacromoléculas, Instituto de Química. [3]Departamento de Fisicoquímica, Facultad de Química. Universidad Nacional Autónoma de México, Ciudad Universitaria. Ciudad Universitaria, 04510, Ciudad de México, México. Phone number: +52 5556232259. E-mail: fdaniel@unam.mx (DAFV)

Protein folding is one of the most fascinating molecular transformations. The spontaneity of this self-assembly process is quantified in terms of the conformational stability of the folded state (ΔG_F). The "stability curve" of proteins, describes the non-linear and strongly temperature-dependent change of ΔG_F as a function of temperature (ΔG_F (T)). Two of the main descriptors of ΔG_F (T) are the maximum in the stability curve (ΔGF_{Max}) and the melting temperature (T_m), related to the "thermal stability" of the protein. The thermodynamic properties of all the non-covalent interactions observed in the native structure of proteins are known. This information has been frequently used by protein engineers to increase either T_m or ΔG_{FMax}. However, we are not able to predict the stability curve of a particular protein using structural data or to modify rationally both T_m and ΔG_{FMax} .

The number of stability curves reported in the literature is three orders of magnitude lower than the number of three dimensional structures in the PDB. Due to this shortage of thermodynamic information, or to other unknown factors, it has not been possible to map the evolution of the temperature adaptation of proteins in terms of their stability curves. Here, we will present our experimental data on the stability curves of proteins that contain one of the most versatile and common protein architectures observed in nature, the "TIM barrel" fold. We found that natural TIMs from mesophilic organisms show a drastic variation in their stability curves, changing both T_m and ΔG_{FMax} with slight structural differences. Regarding protein engineering, we have designed *de novo* TIM barrels with T_m values higher than 115 ^{0}C. Nevertheless, we have been unable to increase ΔG_{FMax} to the values observed in natural proteins. Finally, we will sketch how the study of proteins from phylogenetically-related extant species, those generated by ancestral protein reconstruction and the ones obtained by computational protein design, can contribute to our understanding of the evolution of temperature adaptation in proteins and the rational modification of their stability curves by structural thermodynamics.

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ROLE OF MITOCHONDRIA ON THE CANCER CHEMOPREVENTION BY AN ADENOSINE DERIVATIVE IN A MODEL OF CIRRHOSIS-HEPATOCELLULAR CARCINOMA INDUCED BY DIETHYLNITROSAMINE IN RATS.

Chagoya de Sánchez V., Chávez E., Velasco-Loyden G., Lozano-Rosas MG., Pérez-Carreón JI., Rodríguez-Aguilera JR.
Instituto de Fisiología Celular, UNAM. Circuito Exterior s/n Coyoacán, Ciudad Universitaria, C.P. 04510. Ciudad de México.
Correo electrónico: vchagoya@correo.ifc.unam.mx

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world. It is a complex pathology associate with chronic liver disease like cirrhosis from diverse etiology. The mortality rate is very high mainly because there is not early detection neither efficient treatment. Previously, we have shown that the adenosine derivative (AD) reverses carbon tetrachloride induced cirrhosis and modulate the energy state. We were interested in evaluate its effect in hepatocarcinogenesis induced by diethylnitrosamine (DEN) in rats which was preceded by cirrhosis. Rats were injected with DEN (50mg/Kg) for 12 weeks to induce cirrhosis and for 16 weeks to induce hepatocarcinogenesis, some groups were treated with the AD at the same time. Cancer progression was studied in rats after the DEN treatment finished; they received for 6 weeks saline solution or AD. At the end of the treatment the animals were euthanized, the liver sections and mitochondria were obtained for the following studies: proliferation, hystologic evaluation, apoptosis, proliferation, expression of liver markers of HCC, GGT and GSTP; markers of the cell cycle in the liver tissue. Mitochondria were isolated and the function was evaluated measuring respiratory cocient, ATP synthesis, complex I and V and membrane potential; the metabolic activity was evaluated measuring aspartate-malate shuttle, isocitrate dehidrogenase activity and levels of lactate and acetyl CoA; dynamic mitochondria was studied quantified by western blot of the following proteins: PGC-1α, Sirt-1, DRP-1 (fission) and MFN2 (fusion) and electronic microscopy studies. The compound AD down-regulated the expression of thymidylate synthase, HGF, and increased the cell cycle inhibitor p27, suggesting a chemoprevention mechanism. In summary, the AD also helped to maintain the normal function, metabolism and dynamic of mitochondria like in non tumoral cells probably through mitoepigenetc changes, suggesting its use as a potential HCC treatment.

Mitochondrial calcium transport and permeability transition in plants

Manuel Gutiérrez Aguilar

Upon calcium overload, inner mitochondrial membranes can shift their permeability properties resulting in mitochondrial bioenergetics disruption. The mitochondrial permeability transition (MPT) pore is one cause of such permeability shift and can be defined as an unselective channel allowing passage of solutes with a 1.5kDa exclusion threshold. One known downstream consequence of sustained MPT pore opening is cell death. Although this process has been extensively studied in mammalian cells, there is also evidence suggesting yeasts and plants harbor MPT pore-like structures. Here we sought to address whether pollen tube mitochondria from *Nicotiana tabacum* harbor a canonical calcium induced-calcium releasing MPT pore under *in vitro* conditions. Our results show the presence of a ruthenium red-sensitive mitochondrial calcium uniporter-like transport system in digitonin-permeabilized pollen tubes with intact and coupled mitochondria. Calcium transport displayed a low rate and high capacity. However, calcium release was observed upon massive calcium overload suggesting MPT onset. Calcium release was insensitive to ruthenium red, decylubiquinone and cyclosporin A. The latter in agreement with previous reports showing a CsA-insensitive MPT pore in *N. tabacum*. However, such calcium release was germination stage-dependent, being inhibited at higher germination times. Our results can contribute to understand the potential role of MPT in pollen tube physiology and mitochondrial homeostasis.

Discovery of novel RNase P inhibitors via an activity-binding-structure Pipeline

Alfredo Torres-Larios*, Ezequiel-Alejandro Madrigal-Carrillo[1], Carlos-Alejandro Díaz-Tufinio[1,2], Hugo-Aníbal Santamaría-Suárez[1], Marcelino Arciniega-Castro[1]
[1] Department of Biochemistry and Structural Biology, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Mexico City, Mexico;
[2] Tecnologico de Monterrey, Escuela de Ingeniería y Ciencias, Mexico City, Mexico
* To whom correspondence should be addressed: Tel.: +52-55-5622-5640; Email: torres@ifc.unam.mx.

ABSTRACT

Ribonuclease P (RNase P), an essential ribonucleoprotein complex, is an attractive target for novel antibacterial drugs. Here, we describe a real-time, fluorescencebased platform that can monitor RNase P activity and rapidly identify inhibitors.

Using this method, we screened a library comprising 2560 compounds on the RNase P holoenzyme and identified four hits. These hits were then assessed using biolayer interferometry with either immobilized P protein or RNA to establish which molecular component of the complex was interacting directly with the inhibitor.

Docking, molecular dynamics, and X-ray crystallographic studies subsequently identified a region of the protein correlated with the perturbation caused by one hit on the binding of the leader. This joint activity-binding-structure strategy allows discovering and gaining insight into the mode of action of potential RNase P targeted compounds.

Common bean microRNAs emerging as regulators of the nitrogen-fixing rhizobia symbiosis

Georgina Hernández*, Damien Formey, José A. Martínez-Rodríguez, Bárbara Nova-Franco, Luis P. Íñiguez, Lourdes Girard, José L. Reyes, Luis Cárdenas, Oswaldo Valdés – López, Alfonso Leija, Sara I. Fuentes, Mario Ramírez

Universidad Nacional Autónoma de México *Centro de Ciencias Genómicas Av. Universidad 1001, Cuernavaca, MOR. 62209, México gina@ccg.unam.mx

The microRNAs (miRNAs) are small non-coding RNA sequences, 21 -24 nt in length, that negatively regulate gene expression post-transcriptionally through sequence complementarity, either via target transcript cleavage or translational inhibition. Plant miRNAs are involved in most, if not all, biological processes. Growing evidence supports the participation of miRNAs in the control of the legume-rhizobia N-fixing symbiosis (Lelandais-Brière et al., 2016). Symbiotic N_2-fixation (SNF) by differentiated bacteroids takes place in the rhizobia-induced root nodules. SNF reduces the cost of legume cultivation and is relevant for sustainable agriculture.

Research from our group has contributed to gain knowledge about microRNAs from common bean (*Phaseolus vulgaris*). Common bean is the most important legume for human consumption; it is the principal source of protein of millions of people, mainly in South American and African countries. Genome-wide analysis of common bean smallRNAs from different plant organ, including nodules, and also from isolated root hairs incubated with purified *Rhizobium etli* Nodulation Factors revealed 442 precursors of miRNAs that generate 271 mature miRNAs, 122 of these being novel miRNAs not previously reported in plants. miRNA target genes were identified by degradome- Subsequent research form our group has led to identify miRNAs that are new protagonists for the control of the common bean-rhizobia symbiosis. Examples of the latter are the following.

The common bean miR172c/AP2-1 node is a key regulator of the *R. etli* symbiosis. High miRN172c results in increased rhizobial infection, expression of nodulation genes, nodulation and nitrogen fixation, as well as decreased sensitivity to nitrate inhibition of nodulation. Decrease of AP2-1, induced by miR172, prevents the repression of early nodulins gene expression and the activation of nodule-senescence gene expression in mature nodules.

The role of the miR319d/TCP10 node in the common bean-rhizobia symbiosis was recently reported. Increased miR319d resulted in reduced root length/width ratio, increased rhizobial infection evidenced by more deformed root hairs and infection threads, and decreased nodule formation and nitrogenase activity per plant. The expression level of *TCP10*, correlated with the level of *LOX2*, jasmonic acid (JA) biosynthetic gene. We propose that in roots/nodules of inoculated common bean plants TCP10 could be the transcriptional regulator of *LOX2* and the miR319d/TCP10 node could affect nodulation through JA signaling.

Understanding plant nutrition for a better human nutrition.

David G. Mendoza-Cozatl
Division of Plant Sciences, University of Missouri, Columbia Mo, USA.

Plants and seeds are the main dietary source of micronutrients (Zn, Fe, Cu, Mn) and also toxic elements (Cd, As) for humans; thus, understanding how plants take up, mobilize and accumulate essential and toxic elements will help developing plants and grains of enhanced nutritional value. Our lab uses cell-specific transcriptomics, functional genomics and *ionomics* to identify transporter proteins that mediate the allocation of essential and non-essential elements within plant tissues, including seeds. So far, we have identified 70+ Arabidopsis transporters preferentially expressed in the vasculature, most of them with unknown function. Using high throughput screening assays using different yeast strains we have successfully identified plant transporters that: (1) induce hypersensitivity to Cd or As, (2) confer tolerance to elevated concentrations of Cd or As, or (3) rescue yeast mutants defective on Fe and Zn uptake. In addition, our lab also focuses on how plants sense essential elements and how impaired sensing leads to hyperaccumulation – or underaccumulation – of several elements in a tissue-specific manner. Our results suggest that leaves play a key role in sensing and allocation of elements within the plant. Our current experiments are directed towards the identification of the transcriptional networks that sense the nutrient status of the plant and mediate the crosstalk between different nutrients.

Regulation of pattern formation in plant embryogenesis by miRNAs.

Stewart Gillmor
Langebio-CINVESTAV, Irapuato, México.

Early embryogenesis is one of the most amazing processes in biology. Fertilization of the egg and sperm produces the zygote, a single cell capable of generating all the different tissue types of the adult organism. By studying embryogenesis, we can learn about fundamental processes that determine cell identity, and the small number of cells in each tissue type makes tracking cell fate and morphology relatively simple. In early embryogenesis of *Arabidopsis thaliana*, all major tissue types of the adult plant are generated in the first 5 days after fertilization. The main interests of my laboratory are a) how pattern formation is regulated in these first five days, and b) zygotic genome activation, the process by which the maternal and paternal genomes of the zygote become transcriptionally active.
In my talk, I will present recent results from my laboratory on the role that miRNAs play in regulation of cell fate determination in early Arabidopsis embryos. miRNAs are small (20-24bp) regulatory RNAs that target mRNAs for destruction or translational repression, based on sequence complementarity. Using next generation RNA sequencing, we have discovered about 40 miRNAs that are expressed in the first few days of embryogenesis. These include highly conserved miRNAs that are known to function at other stages of development, and well as novel miRNAs whose function has never been studied. We have also analyzed the functional importance of miRNAs using mutants in miRNA biogenesis pathways, as well as loss of function of individual miRNA genes. These studies have demonstrated that miRNAs are required for determining the polarity of the zygote, and for the first asymmetric divisions of the zygote. These results are very exciting because they demonstrate that miRNAs promote the very first steps of cell identity specification in embryogenesis. Current work in my laboratory is focused on characterizing the interactions of miRNAs and the phytohormone auxin in early pattern formation. Our work is significant because we are learning more about gene regulation and the gene networks that determine cell identity in plants.

The roles of the *myo*-inositol pathway to ascorbate at conferring plants enhanced growth and abiotic stress tolerance

Argelia Lorence
Arkansas Biosciences Institute, Arkansas State University, USA.

L-Ascorbic acid (AsA, vitamin C) is the most abundant water-soluble antioxidant found in plants. Ascorbate has a wide variety of physiological roles including being an enzyme cofactor, a scavenger of free radicals, and donor/acceptor of electron in the chloroplast. Ascorbate protects tissues against damage caused by reactive oxygen species produced through normal oxygenic metabolism or generated from biotic and abiotic stresses. The *myo*-inositol route to ascorbate involves four enzymes: *myo*-inositol oxygenase, glucuronate reductase, gulonolactonase (GNL), and L-gulono-1,4-lactone oxidase. The first two enzymes and the terminal enzyme in the pathway have been already characterized by the Lorence Laboratory. The third enzyme, GNL, has been characterized in rats and multiple bacteria but not in plants. In this work we will describe the biochemical characterization of novel glucuronolactonase (GNL) in Arabidopsis. We have found that homozygous *gnl* knockouts have lower foliar AsA content compared to wild type controls, and display stunted growth, and chlorotic lesions, indicating the involvement of this enzyme in AsA synthesis and in maintaining a healthy redox balance in the leaves. On the other hand, *At*GNL over-expressers and restored lines (knockouts that have been complemented with the functional enzyme) have elevated AsA content, grow faster, accumulate more biomass, and produce higher seed yields than the corresponding controls. I will also discuss how by combining transcriptomics and phenomics approaches we are gaining insights regarding the mechanisms mediating the increased growth rate, biomass accumulation and enhanced abiotic stress tolerance of our high AsA lines.

Adenoviral Replication Compartments Molecular hubs for viral replication and control of virus-cell interactions

Ramón A. Gonzalez.
Centro de Investigación en Dinámica Celular, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos. rgonzalez@uaem.mx

Adenoviruses are ubiquitous infectious agents associated with respiratory, gastrointestinal and conjunctival infections and can result in extreme morbidity/mortality in immunosuppressed patients. These viruses represent a useful model to study fundamental aspects of host-cell gene expression and they are one of the most common types of viral vectors used for clinical applications. The adenoviral genome encodes three groups of oncogenes that are responsible for establishing conditions in the infected cell that are conducive to efficient viral replication and progeny production. The products of these oncogenes are multifunctional proteins that inhibit anti-viral cellular defenses and simultaneously induce the almost exclusive expression of viral genes. The E1A proteins activate transcription of S-phase genes and inhibit the antiviral response. In turn, the proteins encoded in the E1B and E4 genes are responsible for the selective expression of viral late genes, regulation of tumor suppressors and inhibition of apoptosis. Together, these viral early proteins cooperate to induce cell transformation or the efficient production of viral macromolecules and progeny.

The study of adenoviral oncogenes has shown that their products are implicated in the reorganization of the infected-cell nucleus as they participate in the formation of virus-induced structures that constitute nuclear microenvironments that colonize the cell nucleus. These so called viral replication centers (RC) are the sites where viral DNA is replicated and transcribed, and where postranscriptional processing of viral late mRNAs that are selectively exported to the cytoplasm initiates. Interestingly, formation of viral RC recruit and co-opt cellular defense mechanisms that include components of the DNA damage response (MRN, BRCA1, ATR); tumor supressors (PML, p53); anti-viral response factors (STAT1); and a growing list of cellular proteins that would normally interfere with viral replication. Hence, viral RC serve as hubs to direct viral replication and simultaneously control cellular defense mechanisms.

Using adenovirus-infected normal human cells we have found that formation of RC depends on concerted interactions between the early adenoviral proteins and cellular proteins that are co-opted in these sites. The presence of the E1B and E4 proteins in RC correlates with the efficient replication and expression of the viral genome; concomitantly these viral early proteins are also required to recruit cellular factors that are inhibited during viral replication. These findings suggest that the assembly of RC and the reorganization of the cell nucleus underlie the molecular mechanisms that the E1B and E4 adenoviral oncoproteins regulate to take control of the infected cell during viral replication.

Dynamics of the intestinal virome during the first year of life

Blanca Taboada1, Pavel Isa1, Susana López1, Yarenci Aguado1, Angélica Serrano2, Patricia Morán2,Liliana Rojas2, Horacio Pérez2, Javier Torres3, Cecilia Ximenez2, Carlos Arias1.
1Instituto de Biotecnología, Universidad Nacional Autónoma de México, Av. Universidad #2001, Col.Chamilpa C.P. 62210. Cuernavaca, Mor., MX. 2Unidad de Medicina Experimental, Facultad de Medicina, Universidad Nacional Autónoma de México, C.D.Mx., MX. 3Unidad de Investigación Médica en Inmunología, Hospital Pediatría, Centro Médico Nacional Siglo XXI IMSS, C.D.Mx. MX.
btaboada@ibt.unam.mx. Tel. (777) 329-1612

Human body harbor a great diversity of microorganisms that are part of its own 'ecosystem' called microbiome, which includes bacteria, viruses, fungi and archaea, among others. Each compartments of the body has distinct microbiome and its characterization has made it possible to identify its role in health and disease conditions. All this has been possible thanks to the new generation sequencing technologies (NGS), which allow, in principle, to determine in an unbiased manner the sequence of all nucleic acids present in a clinical sample. Until now most studies that have used this type of metagenomic approach have characterized the bacterial component and little is known about the composition of the virome (viral component).

The studies of the human virome of gastrointestinal tract basically have described the diversity of bacteriophages, which is very different between individuals but, unlike bacteria, is stable in each person over time. It has been shown that factors such as diet, the use of antibiotics, and even hereditary, can play a role in the composition and effect of bacterial virus populations. Some studies have characterized phage populations in newborn children, showing that they have a high diversity during the first days, which decreases with age. Regarding eukaryotic viruses in the intestinal tract, although they are far less characterized, 16 different DNA families and 10 RNA have been detected in gut samples. In healthy children, a longitudinal study of newborn twins showed that, in contrast to phages, the eukaryotic virus diversity has a tendency to increase over time.

In the present work, we presented a project aimed at characterizing by NGS the gastrointestinal virome of a cohort of 10 children followed monthly from birth to 1 year old. So far, samples from 3 children have been analyzed where more than 1000 different virus species belonging to 45 viral families were identified (9 families infecting bacteria, 35 eukaryotes and 1 unclassified). As expected, the majority of the sequences were assigned to viruses that infect bacteria. Interestingly, plant viruses of the *Virgaviridae* family were commonly detected, even in samples from children 15 days old. Other families of eukaryotic viruses frequently identified in all samples were *Anelloviridae*, *Picornaviridae*, *Parvoviridae*, *Genomoviridae* and *Caliciviridae*. We have also been able to assemble complete genomes of several viral species. In conclusion, our study will provide detailed insight into the dynamic of viruses in the gut during early state of life.

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Down regulation of antiapoptotic proteins by Feline calicivirus: perspectives to cancer control.

Ana Lorena Gutiérrez Escolano, Oscar Salvador Barrera Vazquez

Caliciviruses are single stranded and positive polarity RNA viruses. As a result of calicivirus infection, mitochondrial cellular apoptosis is induced, leading to viral dissemination in the host. During feline calicivirus (FCV) infection, we have reported that a reduction in anti-apoptotic proteins survivin and X-linked inhibitor of apoptosis (XIAP) occur, which correlates with subcellular location of the proapoptotic protein Smac/DIABLO from the mitochondria to the cytoplasm and the activation of caspase-3. Inhibition of survivin degradation by the use of lactacystin, a proteasome inhibitor, resulted in a delay in apoptosis progression, significantly reducing virus release, without affecting virus production, in accordance to the role of apoptosis in virus spread. Recently, we found that the leader of the capsid protein (LC) from FCV is responsible for the down regulation of survivin and XIAP, and the apoptosis establishment. Survivin is highly expressed in most cancers and is associated with a poor clinical outcome; thus, beside its role in virus infection, down regulation of survivin mediated by LC will be explored as a cancer therapy.

The nonstructural proteins 3 and 5 from flavivirus modulate nuclearcytoplasmic transport and innate immune response targeting nuclear proteins.

Margot Cervantes-Salazar, Ana L. Gutiérrez-Escolano, José M. Reyes-Ruiz, **Rosa M. del Angel1* (rmangel@cinvestav.mx)**

[1] Departamento de Infectómica y Patogénesis Molecular, CINVESTAV-IPN, México, D.F., México.

Viruses hijack cellular proteins and components to be replicated in the host cell and to evade the immune response. Although flaviviruses have a cytoplasmic replicative cycle, some viral proteins such as the capsid (C) and the RNA dependent RNA polymerase, NS5, can reach the nucleus of the infected cells.
Considering the important roles of NS5 in viral replication and in the control of the immune response, and its striking presence in the nucleus, the possible functions of this protein in some mechanisms orchestrated by the nucleus was analyzed. We isolated and identified nuclear proteins that interact with NS5; one of them, the DEAD-box RNA helicase DDX5 is relocated to the cytoplasm and degraded during infection with DENV, which correlates with its function in IFN dependent response.
Since DDX5 and many other proteins are relocated from the nucleus to the cytoplasm during flavivirus infection, the integrity and function of the main regulator of the nuclear-cytoplasmic transport, the nuclear pore complex (NPC) was evaluated. We found that during DENV and ZIKV infection nucleoporins (NUPs) such as TPR, Nup153, Nup98, and Nup62 were cleavaged/degraded. The protease NS2B-NS3 induces NUPs degradation and it causes a dramatic inhibition of mature mRNAs export to the cytoplasm but not the export of DDX5 protein, which is dependent on NS5. Here we describe for the first time that the NS3 and NS5 proteins from flavivirus play novel functions hijacking the NPC and some nuclear proteins relevant in triggering immune response pathways, inducing a favorable environment for viral replication.

Evolution of regulatory landscapes

José Luis Gómez-Skarmeta
Centro Andaluz de Biología del Desarrollo (CABD), Consejo Superior de Investigaciones Científicas/Universidad Pablo de Olavide, Carretera de Utrera Km1,41013 Sevilla, Spain.

Dynamic gene expression is controlled by *cis*-regulatory information located at the non-coding DNA and is critical for tissue formation during animal development. Moreover, changes of this dynamic are essential for morphological diversification along evolution. In the recent years, it has been shown that the vast amount of regulatory information present in animal genomes is organized in the 3D chromatin structure. This organization forms regulatory landscapes that are necessary to precisely control the expression of target genes. In our laboratory, we are interested in determining how regulatory information and regulatory landscapes change during evolution and what is the impact of those changes in gene expression and morphological novelties. Here I will present a comparative study of the evolution of the regulatory information and regulatory landscapes during the transition between invertebrates and vertebrates. Our results provide a better understanding of the regulatory principles underlying key vertebrate innovations.

Regulatory non-coding RNAs as pathogenic factors in neurodegenerative diseases: a functional genomics approach

Dr. Eulàlia Martí
Associate Professor
Dpt. Biomedicine, University of Barcelona, Spain
Unit Leader
CB06/02/0058 CIBERESP, Ministerio de Ciencia Innovación y Universidades

Different classes of non-coding RNA (ncRNA) modulate gene expression and constitute a crucial layer of biological regulation. ncRNAs show highly regulated and specific expression patterns, and control biological networks in response to diverse stimuli. Strikingly, a large fraction of tissue specific ncRNAs are expressed in the central nervous system and mounting evidence indicates that deregulation of ncRNA pathways underlie pathology in neurological and neurodegeneraltive diseases.

Current work in my laboratory is aimed at identifying ncRNA mechanisms that contribute to the onset and progression of age-related neurodegenerative disorders. The final purpose is to discover ncRNA-gene expression networks underlying neuropathogenic processes and identify pathways for therapeutic intervention. To this end we focus on the identification of ncRNAs perturbed early in the course of the disease and on the evaluation of cell pathways regulated by ncRNAs. In this talk I will discuss sequencing, data mining strategies and functional screening approaches to identify ncRNAs involved in neuronal dysfunction. I will focus on our recent work on ncRNA expression profiling in human brain

samples at preclinical and clinical stages of Parkinson's disease. I will also discuss a high-throughput functional screening to systematically identify ncRNAs with a role in the maintenance of neuronal cell viability and response to oxidative stress, a major hallmark in neurodegenerative conditions.

These approaches led us to propose that overall ncRNA expression profiles recapitulate a particular disease stage very sensitively, with specific deregulated species contributing to pathogenesis.

A multiscale modeling approach to study the evolution of antibiotic resistance.

Ayari Fuentes Hernández

Antibiotic resistance is, arguably, the most urgent problem in public health, although it occurs naturally, misused of these substances has accelerated the appearance of resistant organisms, for these reasons is crucial to understand how bacteria is evolving and adjusting to these environments.
Our understanding of the evolutionary forces driving the adaptation of antibiotic resistant bacteria is mostly based on the assumption of constant, homogeneous environments, these assumptions are far from reality. In this talk, I will focus on discussing how an environment with a heterogeneous temporal structure imposes a dynamic range of selective pressures that can influence the emergence and stability of drug resistance in microbial microcosms. To achieve this goal, we use data-driven mathematical and computational models combined with experimental evolution and single cell microfluidic to study how bacterial populations adapt to unpredictable and hostile environments.

Gene expression regulation during *Drosophila* development

Kasia Oktaba
Cinvestav-Unidad Irapuato

Gene expression is tightly regulated in space and time during animal development. This regulation depends on the interaction between enhancers and promoters of target genes to activate transcription. However, enhancers can be located tens or hundreds of bases away from the regulated promoters. Our laboratory is focused on understanding how these elements, that are encoded in the linear genomic sequence, are organized in the three-dimensional nuclear space. This organization or genome topology is essential for understanding how the genome functions, especially how gene expression is regulated in multicellular organisms. We are studying these phenomena in *Drosophila* with the aim of understanding how tissue-specific gene expression is achieved during embryonic and larval development.

Glucocorticoids in the striatum modulate the consolidation and retrieval of emotional memory

Gina L. Quirarte, Norma Serafín, Cristina Siller-Pérez, Renata Ponce-Lina, Rogelio Pegueros Maldonado, and Roberto A. Prado-Alcalá
Departamento de Neurobiología Conductual y Cognitiva, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus Juriquilla Querétaro
Boulevard Juriquilla 3001, Juriquilla Querétaro, Qro. C.P.76230, México
ginaqui@unam.mx

Extensive evidence indicates that glucocorticoid hormones enhances memory consolidation of emotional arousing tasks, and that the basolateral nucleus of the amygdala (BLA) is involved in regulating such glucocorticoid influences on memory consolidation. Glucocorticoid-induced memory enhancement can be blocked by intra-BLA infusion of ß-adrenergic antagonists. Data from our laboratory demonstrate that corticosterone administration into the dorsal striatum produces an enhancement of memory consolidation that can be blocked by concurrent administration of the β-blocker atenolol into the BLA. Additionally, other studies from our group have described that the activation of glucocorticoid receptors in the medial or lateral region of the striatum hinders or enhances consolidation respectively, depending on the type of memory (spatial or procedural). It is also known that glucocorticoids impair the retrieval of spatial memory and that this impairing effect depends on the noradrenergic system of the BLA. In addition, recent findings from our laboratory revealed that glucocorticoids in the dorsal striatum impair the retrieval of a procedural-like cued water maze task. These findings indicate that noradrenergic activation of the BLA is required for enabling striatal glucocorticoid actions in mediating the enhancing and impairing effects of glucocorticoids on consolidation and retrieval of procedural memory.

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When the striatum initiate a movement who drives it?

Fatuel Tecuapetla Aguilar, Joaquim, Alvez da Silva, Rui Costa & Edgar Hernandez Diaz
Instituto de Fisiologia Celular de la UNAM, Mexico
Champallimaud Centre for the Unknown, Lisbon Portugal

Deciding when and whether to move is critical for survival. The normal function of the striatum and its synaptic inputs has been proposed to be critical for the initiation of movements. While in previous reports we have documented the necessity of the striatal activity for the initiation of movements the specific evaluation of its synaptic inputs has been very difficult to achieve. In this talk I will present recent data from the lab and in collaboration on how two synaptic inputs to the striatum support the initiation of movement.

Dopaminergic inputs: It is well known that loss of dopamine neurons (DANs) of the substantia nigra pars compacta in patients with Parkinson's disease causes deficits in movement initiation. The role of DANs in the control of movement has been traditionally attributed to their tonic activity while their phasic activity has been mainly linked to reward prediction. In a recent study we show that a subpopulation of DANs, which did not overlap with reward-responsive DANs, transiently increased their activity before movement initiation and that Inhibition of DANs when animals were immobile reduced the probability and vigour of future movements.

Glutamatergic thalamic inputs: While the hypothesis postulating that the different thalamo-striatal-projections contribute differentially to shape the functions of the striatum is largely accepted, existing technical limitations have hampered efforts to prove it. In ongoing work we identify that specific connections from the thalamus to the striatum are particularly required for the smooth initiation of movements.

These results support a model where phasic dopamine is required for the initiation of movements and different thalamo-striatal subcircuits are specifically required for the smooth initiation of movements Part of this work received support of CONACyT-Fronteras de la Ciencia: 2022 and DGAPA-PAPIIT-UNAM: IA200815 and IN226517 to FT.

Optogenetic Control of appetite: Deciphering the neurons that promote overeating

Dr. Ranier Gutiérrez
Laboratory of Neurobiology of Appetite
Pharmacology Department CINVESTAV

In this symposium, I will talk about the neuronal circuits that control feeding behavior. In particular about the interplay between the hedonic and rewarding systems that control food intake. Specifically, I will describe the new advances in the field of optogenetics applied to the study of ingestive behavior.

This project was supported in part by Productos Medix 3247 and CONACyT Grants Fronteras de la Ciencia 63 and Problemas Nacionales 464 (to RG).

Energy metabolism dysregulation during aging and its role in synaptic function and integrity

Clorinda Arias
Instituto de Investigaciones Biomédicas. Universidad Nacional Autónoma de México AP 70-228.
carias@unam.mx

Aging is associated with a variety of brain impairments that range from subtle decline of cognitive performance to severe memory loss. In healthy aging slowdown of neurotransmission and reduced synaptic plasticity have been reported. Synaptic dysfunction seems to precede the loss of synapsis and neuronal death that occurs in some age-associated neurodegenerative conditions such as Alzheimer´s Disease (AD). Emerging evidence suggest that neuronal bioenergetics dysfunction may be an early event that compromise neurotransmission and synaptic integrity in aging and AD. Energy supply from mitochondria is essential to sustain metabolic demands and signaling functions at the synapsis. We have analyzed mitochondrial function and morphology and markers of oxidative stress in isolated presynaptic nerve endings from the hippocampus that were exposed to the amyloid-β (Aβ) peptide at different ages. We found an age-related decline in mitochondrial activity, reduced antioxidant contents and increased oxidative stress markers in resting and depolarized synaptic terminals. Ultrastructural changes including an increase in mitochondrial size and a significant reduction of synaptic vesicles contents were also observed. In addition, synaptosomes obtained from 24 month-old rats were more sensitive to Aβ toxicity. We also conducted experiments in isolated presynaptic terminals from vulnerable brain regions implicated in AD pathology from the 3xTg-AD mice at different ages and found aged- and AD-associated mitochondria structural changes, reduced mitochondrial bioenergetics and an increase of the activated form of the fission protein, Drp1. These alterations correlated with changes in the mechanism of neurotransmitter release. Thus, we have explored the possible beneficial effects of mitochondria transplantation to restore the bioenergetics of synaptic terminals.

This work was partially supported by Fundación Miguel Alemán, A.C.

MEXICO & USA: Partners in Scientific Research. Are we doing enough? Are they doing enough?

Salomón Bartnicki-García
Departamento de Microbiología, CICESE (Centro de Investigación Científica y de Educación Superior de Ensenada) Ensenada, Baja California, 22860, México.
646-175-0590, bartnick@cicese.mx.

There are many examples of successful collaboration between USA and Mexican scientists, some sanctioned and supported by agreements between our two governments but many the result of very personal interactions. I will give examples of both. Yet with more than of 60 years' experience in the world of science in both countries, I conclude that the potential for binational collaboration remains largely underutilized. Mexican scientists are not taking full advantage of the opportunities that exist for collaboration with compatible colleagues north of the border. Conversely, I see only limited organized interest among US scientists or agencies for reaching south of the border except in projects that involve Mexico as an object of study. Although it is probably too late, supporting scientific research in the North American subcontinent should have been part of the new NAFTA agreement. A good example of collaboration is UC-MEXUS, a consortium founded in the 1980's to support, however modestly, the collaboration between academics of the University of California and Mexican scholars.

In later years, UC-MEXUS partnered with CONACYT to offer support for binational projects. UC-MEXUS made a major move in the 1990's to create the "United States-Mexico Foundation for Science", it remained active for several years but its mission was diverted and became a foundation (FUMEC) to promote binational enterprises and investment in Mexico, a worthwhile purpose, but support for scientific research was abandoned. I have the following recommendations: 1) Individual Mexican scientists should make decisive moves to contact their counterparts in USA with ideas for a productive interaction and take advantage of the good will of US scientists to establish such collaborations. 2) Mexican scientific societies and academies should promote much closer links with their counterparts in the USA. And probably most significant, 3) A renovated CONACYT should engage the National Science Foundation, the National Institutes of Health, or any other major USA agencies, to create binational projects with adequate financial support following the example of UC-MEXUS but in a major scale.

Endothelial Luminal Membrane-GlycocalyxFunctionalities in Health and Disease
The need for an integrative and short monograph on the subject.

Rafael Rubio,
Univ of Virginia & Univ. Aut SLP, Maureen Knabb, *West Chester Univ*

Decades of intensive research has shown that the *In* situ vascular tree “endothelium” is an assortment of highly heterogeneous regulatory cells whose cytoplasm and cell membranes are fused forming functional units. These units are responsible for very diverse processes, each one constituting deceptively independent fields of basic research and pathologies such as selective solute permeability, blood coagulation, tissue inflammation, atherosclerosis, pus formation, metastasis, foreign cells invasions, and regulation of hormone-dependent and/or flow-dependent of diverse parenchymal functions and metabolism. In blood vessels, in some portions, the endothelial cell monolayer is extremely thin and is not an inert physical diffusional barrier, but a chemically complex and selective dynamic interface for the transfer of diverse physiological molecules and even cells across it.

In situ, the above-mentioned limited list of processes of the endothelial entity are initiated and may even be restricted to the complex endothelial luminal membrane-glycocalyx (ELMG); a dynamic and continuously varying organelle. The luminal endothelial membrane, is assumed a “minimally thin” membrane bilayer, in opposition, is a membrane with densely bound chemically complex structures that stretch up to 1µm (1000 nm) toward the lumen and constitutes a multifunctional unit. The ELMG is either the site of reception of diverse stimuli or/and enzymatic molecular transformations or/and the site of initial signaling that regulates the mentioned processes. Most literature on the endothelial cell focuses on each of the above-mentioned processes constituting seemingly isolated and distinct fields of encapsulated research, with experts in apparently different disciplines. However, the multifunctional properties of some molecules give rise to overlaps of findings; frequently ignored, between the different fields. These missed interrelationships between the fields and their knowledge and awareness would enrich them. These interrelationships need to be defined as an attempt to integrate the basic knowledge of the ELMG.

Since research on the ELMG has evolved into deceptively independent research fields, the time is ripe 1) to selectively harvest what are the basic findings of each field, the solid foundations for future work, and 2) try to define what they have in common, 3) to bring them together and 4) try to functionally integrate them.

This creates the need to: a) describe the basic and well-established knowledge which defines each individual field; b) identify the current concepts, their strengths, weaknesses, and unresolved problems; c) based on newer knowledge and methodologies to propose ways of revising prevalent concepts; and d) integrate areas where overlap is clearly indicated. For this purpose, to have a panoramic perception of the whole field; a basic integrative bird’s-eye view**,** is a necessity.

Scholarly publishing: a case for Open Access

Gabriel Gasque, PhD
Senior Editor
PLOS Biology

We are all familiar with the term “publish or perish”: all academics must continually publish their work to sustain and advance their careers. More basically, however, publishing represents the main way by which scientists disseminate their discoveries and engage with their colleagues and the broader society. We, those who stand for Open Access, believe that the scientific literature should be free and its access unrestricted, which is made technically possible thanks to the Internet.

However, Open Access literature is not free to produce, even if it is less expensive to produce than conventionally published literature. The question Open Access advocates have posed is not whether scholarly literature can be made and should be made free of cost, but whether there are better ways to pay the bills than by charging readers and creating access barriers.

From a simple perspective, Open Access advocates understand the scientific literature as part of the scientific enterprise. In the United States, the Federal Government is still the major funder for basic research, contributing twice as much as industry. This number is even larger for countries with less gross domestic product, such as Mexico, where the industry contribution is significantly lower. This means tax payers are the main funders of most research. Therefore, it seems nonsensical to charge them again for access to the output of the research they have already paid for. Open Access, I believe, is an expression of social justice. In line with this conviction, the US National Institutes of Health, the Bill and Melinda Gates Foundation, the European Commission, the US National Science Foundation, and the Wellcome Trust have made Open Access diffusion mandatory for grantees. However, widespread adoption of Open Access practices has not yet been achieved.

In this presentation, I will discuss the strengths, weaknesses, and challenges of Open Access and Open Science in general.

Xochcalco: In the house of the flowers,Jorge L. Folch Mallol

Xochicalco is a pre-Hispanic archaeological site in the state of Morelos. Its name in Nahuatl means "in the house of Flowers" (Xochitl= flower; Calli= house); Co=locative: “in”). The site was founded probably by people leaving Teotihuacan after its decline around the year 600 A.D. A civil war near the 900’s A.D. took place and the site remained abandoned for more than one thousand years until the first explorations took place around 1910 (although its’s ruins had been visited by Alexander von Humboldt in the early 1800’s).
Besides its beautiful landscapes and monuments, Xochicalco has a very important role in paleoastronomy. It was here that astronomer-priests from different parts of Mexico gathered together to unify the Meso-American calendars (Mayan, Zapotec, Aztec, etc.).
Of the main attractions figures the Temple of the Feathered Serpent (Quetzalcoatl) that has fine stylized carvings of that deity in a style which indicates influences of Teotihuacan and Mayan art. In this temple two stories are told: one about the military nature of these culture that conquered many other tribes ensuring the transit from the Pacific Ocean to the Gulf of Mexico. The conquered tribes had to pay tribute to Xochicalco in the form of goods (cotton, cocoa seeds, corn, obsidian, etc.). In the lower body of the pyramid the priest-astronomers talk about the rearrangement of the calendar until they could calculate even step years in the solar calendar.
Xochicalco has three ball games that can be visited. These were ritual games played with a rubber ball that could weigh up to 3 Kg. There were different ways to play these games and this is reflected in the form of the court, although they have the same basic structure in all Mesoamerican cultures, a shape of a capital . However, the slopes and the “goal” differ in each of the three ball games in Xochicalco that were devoted to opposite contraries such as dryness and water; day and night, life and death, etc.

The other astonishing thing about Xochicalco is its observatory. It is unique of its kind and in any other part of the world such a structure can be visited, although there are similar observatories in Teotihuacan and Monte Alban (Oaxaca). Unfortunately, the latter are not open for the public. The observatory is an artificial cave built to allow the study of the movement of the sun, the moon and the planets. The cave walls were covered with stucco and painted black, yellow and red representing the infraworld (the same colors that we see at sunset (“the sun was dying”). The observatory itself consists of a tube that measured from the base to the surface its 8.7 meters high, and which is hexagonal all the way down to its end in the roof of the cave. The chimney has a four-degree angle allowing the sun's rays to be to projected on the floor of the cave from 30 April to 15 August. In the sun's movement towards the Tropic of Cancer and upon its return, respectively, on 14/15 May and 28/29 July, the sun is at its zenith and the astronomical noon, the beam of light falls directly through the chimney showing the image of the sun on the floor of the cave. At noon of the 21 of June, day of the summer solstice, the sun enters the cave without touching the walls of the tube forming a perfect circle in the floor ofthe cave.

Dioxygen Avoidance Theory: An experimentally validated mathematical model for ROS-mediated cell differentiation

Wilhelm Hansberg[1] and Elisa Domínguez-Hüttinger[2]

[1]Departamento de Biología Celular y del Desarrollo, Instituto de Fisiología Celular y

[2]Centro de Ciencias Matemáticas, Universidad Nacional Autónoma de México, UNAM.

Circuito exterior s/n, Ciudad Universitaria, Colonia UNAM, Delegación Coyoacán,

CdMx 04510, México. Tel: +5255 5622-5655, whansberg@ifc.unam.mx

We will first critically review the current dominant paradigm of cell differentiation, which assumes that morphogenetic transitions are a consequence of the unfolding of a predefined gene expression program. Based on experimental and theoretical considerations, we will argue why this paradigm is untenable. We will present a possible alternative paradigm. Using *Neurospora* conidiation as a model-system, we will provide experimental data that supports our notion of cell differentiation as a response to oxidative stress. We propose that the transitions between different cell differentiation stages are determined by oxidative stress. Our data shows that a hyperoxidant state precedes each one of the three morphogenetic transitions that lead to conidia development. As a consequence of a hyperoxidant state, fungal cells differentiate and thereby become increasingly insulated from environmental dioxygen. Based on the integration and analysis of our experimental evidence, we propose a mathematical model to test our Dioxygen Avoidance Theory. Using a hybrid system of ordinary differential equations, we mathematically represent the regulatory interplay between the critical biomolecular players that mediate microbial cell differentiation in response to oxidative stress: (1) Intracellular dioxygen, (2) Reducing power, (3) Reactive oxygen species, and (4) Barriers to oxygen, which are produced during the differentiation stages. Dynamic simulations of our model show that the progressions between the different cell differentiation stages are preceded by transient episodes of oxidative stress. Mathematical analysis of our model show that the transient rises in oxidative stress induce differentiation events through a Boundary Equilibrium bifurcation, which leads to the switch-like emergence of new, more differentiated states that further decrease the oxygen permeability. Given the reversibility of these bifurcations, our model can also describe the return to the growth state from any differentiated (insulated) state that is observed in experiments. Our experimental and mathematical results provide arguments in favor for the Dioxygen Avoidance Theory.
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Influence of redox signaling on the regulation of the Erk1/2 kinase and activation of the transcription factor Nrf2 in hearts with post-conditioning.

Macías-López Arturo, Jorge Díaz-Ruiz, José Guevara-Chávez, Alcalá-Vargas Francisco and Buelna-Chontal Mabel Instituto Nacional de Cardiología Ignacio Chávez Juan Badiano #1, Sección XVI 14080, Tlalpan, Mexico City. Tel.: 55732911 Ext. 1465; e-mail: mabel.buelna@comunidad.unam.mx

Ischemic heart disease is the leading cause of death in the world, it is necessary to develop strategies to preserve cardiac function. Since reperfusion is the inescapable strategy applied to restore the blood flow to ischemic tissue, it is paradoxically in turn the event that damages heart the most in phenomena known as reperfusion injury.

Post-conditioning (PostC) has shown to be among the most effective protection strategies in preventing reperfusion damage. This protective mechanical maneuver has shown remarkable success when used as a treatment against acute myocardial infarction, mainly because it diminishes the exacerbated production of reactive oxygen species (ROS) and nitrogen (RNS). Another of the mechanisms activated by PostC, is the activation of survival kinases such as Erk1/2, so they help maintain the viability of re-perfused myocardium. Also, we have found that PostC increases the induction of the expression of enzymes of phase 2 or detoxificants and antioxidants, directed by the transcription factor Nrf2, which is responsible for the maintenance of redox homeostasis important in cardioprotection by PostC.

Recently, a possible role of ROS in the activation of these protective mechanisms has been raised, however, the mechanisms or the targets involved in these are still not well understood. ROS are recognized signaling molecules that may be involved in survival responses (redox signaling). Due to their chemical nature, H_2O_2 and nitric oxide (NO) have been designated as the main redox signal transmitters. The objective of this project is to study whether the cardioprotection triggered by the PostC depends on the redox signaling and whether it regulates the participation of survival kinases such as Erk1/2, as well as the activation downstream of the transcription factor Nrf2 to contribute to the maintenance of the redox balance during long reperfusion. Our results show that the protection activated by the PostC in the ischemic myocardium is blunted by the treatment with ascorbic acid (asc), which suggests that the mechanisms of cardio protection are related to redox-dependent signals. We quantified H_2O_2 and found that it is markedly diminished by the effect of asc in hearts with PostC. In these pro-reductive conditions, we evidenced the decrease of the activation of Erk1/2 and downstream Nrf2 nuclear accumulation, because of the inhibition of the redox signaling in the iPostC by the asc, which affected significantly the cardiac function during reperfusion. On the other hand, we have found that the redox-dependent activation of Erk1/2, does not occur because of a direct oxidation (redox-reversible) on the kinase. In conclusion, our results suggest that the protection triggered by the PostC involves a response that promotes redox-dependent signals, which in turn are related to the activation of the Erk1/2 pathway and downstream the positive regulation of the transcription factor Nrf2 to maintain the redox balance in long-term reperfusion.

Transcriptome analysis of the antagonistic effect of NADPH oxidase gene *RbohB* in the common bean after rhizobial and arbuscular mycorrhizal inoculation

Citlali Fonseca-García[1], Alejandra E. Zayas[1], Jesús Montiel[2], Noreide Nava[1], Enrique Merino, Marco Juárez, Federico Sánchez[1], Carmen Quinto[1]

[1]Departamento de Biología Molecular de Plantas, Instituto de Biotecnología, UNAM, Cuernavaca 62271, Morelos, Mexico.
[2]Centre for Carbohydrate Recognition and Signaling, Department of Molecular Biology and Genetics, Aarhus University, Aarhus C 8000, Denmark.
Corresponding author: quinto@ib.unam.mx; Tel.: +52-777-329-1642

The reactive oxygen species (ROS) mainly generated by NADPH oxidases (called Respiratory Burst Oxidase Homologs (RBOHS) in plants) are involved in numerous plant cell signaling processes, and have critical roles in the symbiosis between legume and nitrogen-fixing bacteria and arbuscular mycorrhizal fungi (AMF). Studies in our group indicate that, down-regulation of *PvRbohB* in *Phaseolus vulgaris* impairs rhizobia infection thread (IT) progression and nodule development, but also enhances the size of appresoria and AMF hyphal colonization. An opposite effect was observed in *P. vulgaris* roots overexpressing *PvRbohB*, where rhizobial infection and nitrogen-fixing activity were enhanced, while AMF invasion was significantly abrogated. These data strongly suggest that *PvRbohB* exhibits an antagonistic mechanism that differentially regulates contrasting features between these mutualistic symbioses. To further explore the impact of *PvRbohB* silencing, the transcriptome of *P. vulgaris* roots under control conditions (transgenic roots generated by *A. rhizogenes* K599) and *PvRbohB*-RNAi conditions inoculated or un-inoculated with rhizobia (*R. tropicii*) or AMF (*R. irregularis*) were performed through RNA-Seq on the HiSeq 2000 platform.
The transcriptome analysis showed that at 7 dpi, 2,741 genes were differentially expressed with rhizobia using a cutoff threshold of ≥1.5 Log2FoldChange and a FDR or P-adjusted ≤ 0.05. By contrast in AMF roots, only the expression of 540 genes was significantly modified, of which 152 were shared between both symbioses. These results indicate that root nodule symbioses induce greater transcriptome reprogramming than AMF in *P. vulgaris* transgenic roots. Additionally, the transgenic *PvRbohB*-RNAi roots inoculated with *R. tropici* have shown 1328 differentially expressed genes, while under mycorrhization conditions only 307 differentially expressed genes were observed. These are approximately the half of the number of genes observed with differential expression in the control samples under nodulation and mycorrhization conditions. The functional annotation indicated that there is a compendium of differentially expressed genes related to ROS-scavenging, auxin homeostasis, cell growth and cell wall-remodeling during nodulation and mycorrhization in *P. vulgaris* that were affected by *PvRbohB* silencing. These results provide relevant information on symbiotic gene signaling networks that respond to rhizobial and mycorrhizal colonization at early stages and the antagonistic effect that NADPH oxidase gene *RbohB* has in these processes.

A LORELEI characterization during the *Rhizobium*-legume interaction

Pascual Morales Edgar J., Cesario Solís Mariana, Quinto Carmen, and Cárdenas Torés Luis
Instituto de Biotecnología/Dpto. Biol. Mol. de Plantas., Universidad Nacional Autónoma de México.
Tel. 52+777-3290872, 777-3291666 fax. 777-3136600 edgarp@ibt.unam.mx

Reactive oxygen species (ROS) in plant cells play an important role in several physiological processes, for example: in plant development, biotic and abiotic interactions, hormonal signaling, polar growth, reproduction, etc. It is well known that EROs represent a dual role during stress and development processes, it means that the concentration, dynamics and subcellular distribution could have antagonistic responses in a similar way to what has been described with intracellular calcium. In plant cells, the biogenesis of ROS has been widely linked to the NADPH oxidase activity. However, the activity of NADPH oxidase depends from other molecular interactors acting upstream the signaling cascade. For instance, FERONIA (a receptor like kinase) has emerged as an important regulator of the NADPH oxidase, which regulates the localized production of ROS at the apical pollen tube and root hairs. Both FER and NADPH oxidases are localized in the apex of the root hair, where lipid rafts domains have been described. These membrane microdomain are sterols enriched regions and contains some proteins modified with a glycosyl-phosphatidyl-inositol (GPI). In *Arabidopsis thaliana*, mutation in *llg1-2* a LORELEI like protein (LGPI) which has a GPI motif results in a phenotype very similar to *fer4* and affects ability of FER to localize in the plasma membrane, therefore, FER is sequestered in the endoplasmic reticulum. Since FER requires LGPI protein for a correct targeting to the plasma membrane, we have functionally analyzed them in *Phaseolus vulgaris* and evaluate their role in the mutualistic interaction. We have characterized at least one LGPI which significantly accumulates in mature root nodules, suggesting a functional role during nodulation. The subcellular localization and their responses during the different stages of the nodulation will be presented and discussed.

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Global gene expression analysis reveals a persistent activation of NRF2 in liver carcinogenesis: Implications in cancer drugs resistance.

Julio Isael Pérez-Carreón[1], Carlos David López-Torres[1], Dafne Guerrero-Escalera[1], Paulette Castro-Gil[1], Saúl Villa-Treviño[2], Julia Torres-Mena[1], Norma Arely López-Hernández[1], Jaime Arellano-Robledo[1].
Laboratorio de Enfermedades Hepáticas. [1]Instituto Nacional de Medicina Genómica, INMEGEN. [2]Biología Celular del CINVESTAV.
Correspondence at www.inmegen.gob.mx Tel 5350-1900 ext. 1170.
jiperez@inmegen.gob.mx

Background: There is no curative treatment for liver cancer through pharmacological strategies because the neoplastic cells acquire chemoresistance ability. The cellular resistance mechanisms may include an exacerbated metabolism of drug detoxification in which NRF2 is a critical transcription factor.

Aims: To clarify the molecular mechanisms that confer cellular resistance, we analyzed the temporal change of global gene expression, in a described model of hepatocarcinogenesis in rats.

Methods: Animals were injected with the carcinogen diethylnitrosamine once a week for up to 16 weeks. Euthanasia was implemented in groups of 6 animals at 6, 12, 18 and 22 weeks. The liver was preserved and nodular lesions were identified by the expression of the tumor markers GGT and GSTP. Total RNA was extracted from liver and the global transcriptome was analyzed using Affymetrix microarrays.

The differential gene expression was calculated and validated for some genes using several methods such as RTqPCR, Western blot, immunofluorescence microscopy and chromatin immunoprecipitation (ChIP).

Results: Liver damage was evident from week 6 and fibrosis was observed at week 12, multi-nodular carcinomas were evident from week 18 and prevailed until 22. Despite the progressive pathological course, the enrichment analysis showed three main cellular processes in the four evaluated times of tumor progression: 1) The Keap1 / NRF2 pathway, 2) Metabolism of Glutathione and NADPH and 3) Cell cycle. The balance of gene expression denoted a switch in drug metabolism genes a decrease in phase I and increase in phase II. We generated a profile of 15 NRF2 target genes that contribute to resistance to drugs such as Nqo1, Akr1B10, Gclc, Gclm, and Ptgr1, the high expression of these genes was confirmed by RTqPCR and by Western blot. The NRF2 protein was localized in the nucleus of the neoplastic cells and the interaction of NRF2 with its target genes Gclc, Nqo1 and Ptgr1 was determined by chromatin immunoprecipitation confirming the persistent activity of this transcription factor.

Conclusion: Genomic research in a model of hepatocarcinogenesis allows to delineate the temporal course of pathology and of pathways such as KEAP1 / NRF2, which is transcendental in the pharmacological resistance of hepatic tumors. The modulation of the factor could offer opportunities for sensitization in the pharmacological treatment of liver cancer.

Development of monitoring oxygen and carbon dioxide transfer rate (OTR and CTR) device and cotton closure simulation during *Pichia pastoris* shake flask cultures

Daniel Cabrera Santos (danielcabsantos@gmail.com); Mauricio A. Trujillo Roldán (C.P. 04510; Tel. 56229192; maurotru@gmail.com).

Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México.

The methylotrophic yeast *Pichia pastoris* has been established as one of the most popular platforms for recombinant protein production due its advantages against other prokaryotic systems (i.e. very efficient secretion system, capability for postraslational modifications and innocuity for human health). Although there is a plethora of surveys dealing with the effect of operational variables over stoichiometric parameters of *P. pastoris* cultures, there is little information about the effect of oxygen and carbon transfer rate. In this topic, the obtained results reveal the lack of clear trends, suggesting that the effect of oxygen is a protein or strain dependent phenomena that could be monitoring by means of respirometric parameters as oxygen transfer rate (OTR), carbon dioxide transfer rate (CTR) and respiratory quotient (RQ). In this work we develop a device for monitoring the OTR, CTR and RQ of *P. pastoris* X-33 strain shake flask cultures. Then, to test our device, we measured and simulated the coefficient diffusion of cotton closures during *P. pastoris* cultures monitoring OTR, CTR, RQ and DOT and compare with conventional cotton closed shake flasks cultures in terms of DOT evolution, optical density and specific growth rate (μ). The reliability of our system was tested with glucose as carbon source, the results agree well with that obtained with RAMOS system.

Our system comprises a valves system which supplies air satisfying the measured coefficient diffusional values, probes for oxygen and carbon dioxide concentration in the gas phase (located in the shake flask headspace) and a device for dissolved oxygen tension (DOT) measure in liquid phase as well as equations for calculation of OTR, CTR and RQ from concentration data. Then, we measure the diffusion coefficient of three different closures: conventional cotton closures (mass=5.9 g, vol=62.8318 cm^3, ρ=0.0939 g/cm^3), 0.00853±0.00145 mol O_2/h; microfiber filter with pore size of 0.2 μm, 0.00646±0.00049 mol O_2/h and PTFE filter, 0.0163±0.00049 mol O_2/h. The measured diffusion coefficient of cotton closure, which corresponds to airflow of 0.018 L/min, was chosen for simulation with the valve system. The DOT behavior was very similar in cotton closure simulation and closed with real cotton closure cultures, starting oxygen limitation conditions at the 8 hours and extending for about 60 hours. Importantly, statistical analysis indicated that it doesn't exist differences in terms of specific growth rate: 0.2214 y 0.2427 h^{-1}, for real cotton closed cultures and simulated cotton cultures, respectively.

We found that the OTR range values: 40-60 mmol/L h and CTR range values: 40-50 mmol/L h, indicated an oxygen limitation process observed as a typical increase-plateau-decrease shape plot described in other works. The RQ range value of 0.80-0.85 agrees with theorical values of glycerol (0.85). Finally, the reliability of our system was tested with glucose as carbon source, we observed the same metabolic response observed during *P. stipitis* cultures with RAMOS system.

Zinc dependent and HIZR-1 mediated activation of HLH-30 and the autophagy-lysosomal pathway is required for zinc homeostasis

Ciro Cubillas, Daniel Schneider, and Kerry Kornfeld

Department of Developmental Biology, Washington University School of Medicine, Campus Box 8103, 660 South Euclid Avenue, St. Louis, MO, USA, 63110. (314) 747-1480
Corresponding author: kornfeld@wustl.edu

Zinc is an essential metal ion for all metazoans but its intracellular concentration imbalances can lead to disease. In animals, zinc is stored in either vesicles called zincosomes or in lysosome related organelles called bilobed granules. In order to maintain zinc homeostasis under fluctuating metal levels, zinc regulated transcription is necessary.

HIZR-1 is a zinc-sensing nuclear receptor that acts as a transcriptional activator in *C. elegans* constituted by a ligand binding domain which binds zinc, and a DNA binding domain which binds a DNA enhancer element present in most zinc-inducible genes called the high zinc activation (HZA) element. Under high dietary zinc conditions, HIZR-1 transcription is activated and zinc-storing structures called bilobed granules are formed in the *C. elegans* intestine cells. Although the connection between these two events is not completely understood both are necessary to survive under high zinc conditions.

Our main goals are: 1) Define the *Caenorhabditis elegans* zinc regulon, the set of zinc activated and repressed genes, through the use of genome-wide gene expression (RNA-sequencing) and bioinformatics approaches and 2) Establish how zinc driven transcriptional activation promotes the formation of bilobed granules.

Here we describe a positively regulated zinc network controlled by HIZR-1 and the master regulator of the autophagy-lysosomal pathway in *Caenorhabditis elegans* called HLH-30. The HIZR-1 and HLH-30 activate the expression of many lysosomal genes under high dietary zinc conditions, some of which are crucial components of the bilobed granules.

Our studies indicate that the HIZR-1/HLH-30 network is crucial for connecting zinc homeostasis to lysosome biogenesis, as it controls the autophagy-lysosomal pathway in *C. elegans*. Accordingly, under high zinc conditions both *hizr-1* and *hlh-30* increase their transcription and accumulate in the nucleus of intestinal cells. Mutants in either *hizr-1* and *hlh-30* are unable to grow under high zinc conditions indicating a critical role for zinc stress tolerance. We also found that the canonical autophagy proteins LGG-1 and SQSTM-1 colocalize with CDF-2, a zinc transporter and structural component of the bilobed granules, as well as with the fluorescent zinc probe fluozin-3.

Since zinc cannot be degraded, the autophagy-lysosomal pathway emerge as a key process necessary to remove either the zinc excess or necessary to cope with the damage caused by the high concentrations of labile zinc.

Quantitation of the relative protein levels of *Debaryomyces hansenii* catalases A and T through their fusion to a fluorescent reporter.

Brandon Bustos-García, Claudia Segal-Kischinevzky, Viviana Escobar-Sánchez.

Laboratorio de Biología Molecular y Genómica, Facultad de Ciencias, UNAM
Circuito exterior s/n, Ciudad Universitaria CP 04515, Cd. Mx. Tel/fax 56 22 48 31,
email: BrandoM@ciencias.unam.mx

Catalases are enzymes present in almost every organism, prokaryote or eukaryote, exposed to oxygen. These enzymes have the ability to degrade hydrogen peroxide, a ROS, down to oxygen and water.

Debaryomyces hansenii is an euryhaline yeast able to use a variety of nonfermentable carbon sources like methanol, ethanol and glycerol. It has two catalaseencoding genes, *Dh*CTA and *Dh*CTT, whose products show catalase specific activity that is several-fold higher compared to the homologous catalases of the budding yeast *Saccharomyces cerevisiae*. However, since *D. hansenii* cannot be genetically modified by the currently available procedures, an acatalasemic strain of *S. cerevisiae* is used as experimental model.

In previous studies we have proved that the heterologous expression of *D. hansenii* CTA and CTT in *S. cerevisiae* is beneficial for growth under different culture conditions. Nevertheless, one of the major challenges of this strategy is that the expression profile on the transformed strains is not always stable, as noticed by catalase-activity assays.

As an alternative approach, we designed constructs with each catalase fused to a fluorescent protein (YeCitrine) in order to determine the relative protein levels by confocal microscopy. Strains were grown in media with either glucose or ethanol as carbon source, and with or without NaCl in both cases.

The strains carrying either the tagged or the non-tagged catalases have shorter doubling times under the different culture conditions compared to the acatalasemic strain; furthermore, the fluorescently tagged strains present the fastest growing rate.

Additionally, the strains transformed with the tagged catalases exhibit higher catalase-specific activity when it is detectable by the method used. Moreover, there appears to be a direct correlation between the relative protein levels detected by confocal microscopy and the specific activity profile of the strains in each medium.

"Analysis of the *Physcomitrella patens* methylation machinery mutant on development and stress response"

Laura Esther Estrada Martinez[1], Jose Luis Reyes Taboada[2], Miguel Ángel Villalobos López[3], Catalina Arenas Huertero[1,4.]
Posgrado en Ciencias en Bioprocesos, Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí[1]. Instituto de Biotecnología, Universidad Nacional Autónoma de México[2]. Centro de Investigación en Biotecnología Aplicada, Instituto Politécnico Nacional[3]. Facultad de Ciencias, Universidad Autónoma de San Luis Potosí[4]. Diagonal Salvador Nava Mtz s/n, Zona Universitaria, San Luis Potosí., SLP, México. CP 78290. catalina.arenas@uaslp.mx.

Chemical modifications upon nucleotides of RNA accomplish a new layer on gene regulation upon the transcriptome, "the epitranscriptome", making the equivalent of the epigenome on DNA nucleotides and histones. N^6-metiladenosine (m^6A) is an epitranscriptomic mark which happens to be very important because it participates in different aspects of RNA metabolism, including biogenesis, translation, stability, degradation and more, making this modification a key point of gene regulation. The machinery involved in this process is still in study, it is composed by three main protein groups named "erasers" which are the demethylases, "readers" which can interpret the methylation label and direct the RNA´s fate, and "writers" which are the methyltransferases. One of these writers in mammals is Methyltransferase like 3 (METTL3) which contains the methyl transferase activity. A phylogenetic analysis of the METTL3 gene showed that this sequence is highly conserved among many eukaryotes such as yeast, plants and *Drosophila melanogaster* due to its role in essential physiological functions such as cell cycle and embryonic development. Accordingly, the knockout of MTA (the METTL3 homologue in *Arabidopsis thaliana*) causes embryonic lethality. We are interested in studying this machinery, specifically the METTL3 homologue in *Physcomitrella patens* (PpMETTL3) under normal conditions and its role in the response to abiotic stress. We are characterizing the phenotype of a *Ppmettl3* mutant (generated by CRISPR-Cas gene editing) and its behavior under adverse conditions. Observations showed the lack of deleterious phenotypes affecting the survival of the *Ppmettl3* mutant in vegetative development stage under normal or stress conditions, but we have observed a significant difference in the rate on protonemal tissue differentiation between WT and *Ppmettl3* mutant where we noticed a probable accumulation of caulonemal *versus* chloronemal tissue (this are the two different types of protonemal tissue in *P. patens*). The mutant and WT cultivated on soil both developed sexual organs and spores indicating no affection on the reproductive stage. We also evaluated the gen *Pplea3* which is positively regulated under salt and ABA treatment and we noticed a deregulation of this transcript on the mutant indicating that it may be regulated by *Ppmettl3*. We are looking forward to evaluate a set of genes involved on development and differentiation that could be regulated by *Ppmettl3* and for that might be dysregulated in the mutant causing the phenotype we have observed on development assays.

Inaccurate microRNA detection in every cell culture due to Fetal Bovine Serum

Carlos Fabián Flores-Jasso, Mariana Flores Torres, Selma Eréndira Avendaño-Vázquez.
Instituto Nacional de Medicina Genómica
C.P. 14610, Phone +52 (55) 5350-1900 ext. 1138. cfflores@inmegen.gob.mx

microRNAs are small, non coding RNAs that silence gene expression byhybridizing partially to the 3'–UTR of target mRNAs. It is estimated that microRNAs can silence up to 70% of the coding genes in the human genome; which makes them key elements in the gene expression regulatory network. Their regulatory function and most basic molecular mechanisms have been uncovered in great extent by the use of cell cultures in which microRNA dependent gene expression is measured, or altered, by using a 3'–UTR fused to a reporter gene under controlled conditions.

Fetal bovine serum (FBS) is widely used in the life sciences as a supplement for cell culture media. It was discovered recently that in all biofluids of mammals, including serum, there are circulating microRNAs (among other molecules) that may serve as biomarkers for the early onset of several diseases. Some of the microRNAs in the biofluids are contained inside exosomes —extracellular vesicles ranging within 30–100 nm in diameter. Apart from being secreted practically by all animal cell types studied so far, exosomes possess an important feature: they can transfer and deliver their cargo into recipient cells, both *in vitro* and *in vivo*. An example for this is the delivery of exosomal microRNAs, which maintain their silencing effect on target mRNAs into recipient cells after their transfer. For this reason, exosomes are the focus of many laboratories interested in exploiting their delivering capability to use them as delivery vehicles for drugs and other molecules.

Since FBS is a biofluid, and therefore, contains a large amount of exosomes, we asked whether it would be possible that bovine microRNAs contained in FBS exosomes, could be transferred into cell cultures as if they were recipient cells. If this could be the case, it would imply that published research carried out in cell cultured under FBS addition, and aimed to measure small RNAs, might contain bovine microRNAs. Since most microRNAs are conserved (or identical) between cow and, for example, humans, it would be impossible to discriminate between them with the current technology. Depending on the delivery extent, conserved bovine microRNAs might even have a functional effect into the recipient cells, masking the true silencing effect of intracellular microRNAs over mRNA

expression. Here, I present the results of a comprehensive meta-analysis that comprises more than 2000 samples of high-throughput sequencing, 80 different types of primary cultures and more than 40 types of cell lines of mice and humans, aimed to answer whether bovine RNA is transferred into cell cultures due to supplementation with FBS.

Optogenetics applied to PLK4, the master regulator of the centrosome

Eduardo Brito-Alarcón [(1)] , Rodrigo Alain Migueles Ramirez [(1)] ,
Chris Wood (1) & Adán Guerrero [(1)] .
[(1)] Laboratorio Nacional de Microscopía Avanzada, Instituto de Biotecnología, UNAM
Correspondence author: Adán Guerrero Zip Code: 62210
Phone: +52 (777) 329-1867 e-mail: adanog@ibt.unam.mx

The centrosomes are non-membranous cellular organelles whose function is to segregate equitative each set of chromosomes during cell division. PLK4 is the master regulator of the centrosome. In the absence of PLK4, there won't be new centrosomes, nevertheless, if there is an PLK4 abundance, it will be active and this will generate in *de novo* genesis of centrosomes. PLK4 is a highly sensitive protein, experiments where PLK4 was controlled under an inducible promoter, the leak transcripcion resulted in *de novo* centrosomes.
It has been reported that for the activation of PLK4, is needed another PLK4.
To our knowledge, there is no evidence to address the state of oligomerization of PLK4 at the centrosome. In our lab, we have approached to this fact with the use of ***F*** *luorescence* ***C*** *orrelation* ***S*** *pectroscopy* (**FCS**) like ***N*** *umber* ***& B*** *rightness* (**N&B**). With this strategy, we can determine (without the need to lyse the cell) the state of oligomerization of PLK4 which came to be *dimers* at the centrosome. Nowadays, we are developing a *plk4-**mRuby3*** $^{+/+}$ cell line, so we can monitor endogenous levels of PLK4. In parallel, we have been designing 3D printed devices to illuminate eukaryotic cell cultures useful for optogenetics, altogether with a CRISPR/Cas9-based photoactivatable transcription system. This set of tools will let us regulate the levels of endogenous PLK4 across the cell cycle and quantify the number of molecules needed for *de novo* centrosome amplification.

Myelin and Lymphocyte Protein (MAL) induces MUC1 lysosomal degradation.

Antonio Roberto Lara Lemus, Bismarck Vázquez Almazán, Olivia Durán, Alejandro Leija and Ana Karina Saldaña Villa.
Depto. de Investigación en Bioquímica, Instituto Nacional de Enfermedades Respiratorias "Ismael Cosío Villegas". Calz. de Tlalpan 4502, México, DF 14080. Tel. (55) 54871705.
betony44@hotmail.com

MUC1 is a hetero-dimeric trans-membrane glycoprotein expressed on the apical surface of mucosal epithelial cells, but it is over-expressed and aberrantly glycosylated in transformed cells. Oncogenic activity of MUC1 relies mostly on MUC1-carboxy-terminal subunit (MUC1-C). After extracellular MUC1-N subunit is released from the plasma membrane MUC1-C is translocated to the nucleus, where it acts as a transcriptional factor, activating genes related to oncogenesis and metastasis. Myelin and lymphocyte protein (MAL), is an essential component of glycolipid and cholesterol-enriched membrane microdomains (lipid rafts) machinery for apical sorting of membrane proteins in epithelial cells. Hyper-methylation of MAL gene silences its expression, and it is a feature found in different adenocarcinomas. On the other hand, transgenic expression of MAL represses the formation of tumors in nude mice, decrease cell motility and induce Fas-receptor mediated apoptosis. However, so far there is no evidence about of the possible mechanisms behind the tumor suppressor activity of MAL. We are interested to determine if MAL could interfere with MUC1-C oncogenic properties. Experimental approach included both generation of different HCC827 cell clones, expressing GFP-MAL and transient transfections. Analyses of phenotypes were made by western blot (wb) and indirect immunofluorescence (IIF). Results showed that MUC1 levels are practically abolished when GFP-MAL is expressed by all 6 isolated clones. Amount of MUC1-mRNA was not affected by MAL expression, but treatment of the cells with lysosome-inhibitor ammonium chloride, rescued the levels of MUC1. It indicates that MAL expression induce lysosomal degradation of MUC1 rather it decrease gene expression. Next we observed a reduction of 8 times in cyclin D1-mRNA levels, associated with expression of MAL. It was perhaps due to lack of MUC1 trasncriptional activation in those cells, indicating that MUC1 degradation associated to MAL expression directly affect transcriptional activation of MUC1 of Wnt pathway-activated genes. These results constitute the first tumor suppressor activity so far described for MAL

Identification of Novel Substrates of Protein Tyrosin Phosphatase 1B in Breast Cancer Cells Through SILAC-based Phosphoproteomics

Luis Enrique Arias Romero[1], Olga Villamar Cruz1 and Marco Antonio Loza Mejía[2]

[1] Unidad de Investigación en Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México. Av. de los Barrios #1, Los Reyes Iztacala. Tlalnepantla, Estado de México, Mexico. C. P. 54090. email: larias@unam.mx
[2] Facultad de Ciencias Químicas, Universidad La Salle Campus Ciudad de México, Benjamin Franklin 47, Cuauhtémoc, México 06140, México

ABSTRACT

PTP1B is a non-receptor protein tyrosine phosphatase that plays a major role in inhibiting signaling from the insulin and leptin receptors. Recently, it has been found that PTP1B is overexpressed in a significant subset of breast and ovarian cancers, and plays an unexpected positive role in HER2 signaling via the direct activation of Src and by the inactivation of p62DOK with the consequent activation of MAPK signaling. However, experiments using cultured cells and mouse models of breast cancer have yielded conflicting results regarding the identity of the key substrates of PTP1B that mediate its positive role in HER2 driven transformation.

In order to identify the immediate direct targets of PTP1B in HER2 positive breast cancer cells, we undertook a SILAC strategy, followed by phospho-peptide enrichment, and quantitative MS. Top canonical pathways that were inhibited upon PTP1B knock down include EGFR, insulin receptor JAK/STAT and MAPK signaling. Interestingly enough, several putative novel targets of PTP1B were identified. For validation, we selected three oncogenes, VAV1, Lyn and CDK3, which are relevant for the initiation and/or progression of different types of cancer.

Molecular docking studies revealed stable interactions between the PTP1B catalytic domain and the aforementioned putative targets. In addition, *in vitro* phosphatase assays confirmed that VAV1, Lyn and CDK3 are dephosphorylated by PTP1B at Tyrosine residues 142, 397 and 15 respectively. Finally, coimmunoprecipitation and co-localization experiments showed that PTP1B interacts with VAV1, Lyn and CDK3 in a cellular context, suggesting novel roles of this phosphatase in the regulation of several cellular processes including cell cycle progression, migration, survival and proliferation.

"Role of pALT$^{Ink4a/b}$ in mouse embryonic fibroblasts during the establishment of cellular senescence"

Miguel del Ángel Muñoz and Susana Castro Obregón

Cellular Physiology Institute, National Autonomous University of Mexico (UNAM), Cto. Exterior s/n, Ciudad Universitaria, 04510 Ciudad de México, CDMX. Phone: 5622 5676. E-mail: scastro@ifc.unam.mx

Abstract

The study of organisms with extraordinary lifespans, and negligible senescence is of great relevance to the evolutionary biology of ageing. One of these remarkable organisms is the Naked Mole Rat (*Heterocephalus glaber*), which outlives other rodents of the same size by more than 30 years, has great resistance to cancer among other age-related diseases and *in vitro*, to cellular senescence. Senescent cells are characterized by a permanent detention of the cell cycle, carried out by cyclin-dependent kinase inhibitors such as p21^{Cip1} and p16^{Ink4a}; there are nuclear aberrations, an elevated activity of β galactosidase, accumulation of lipofuscin and a secretory activity which includes an array of cytokines, chemokines, growth factors and metalloproteases, During ageing, the locus *Cdkn2a/b*, which encodes for the tumour suppressors p16^{Ink4a}, p15^{Ink4b} and ARF, is de-repressed and the expression of p16^{Ink4a} leads to cellular senescence. The accumulation of senescent cells is one of the hallmarks of ageing, due to its secretory phenotype, they alter the tissue architecture and induce inflammation. The elimination of p16^{Ink4a} expressing senescent cells reduces several ageing features. Interestingly, *H. glaber* is the only known animal that expresses an alternative spliced mRNA, called pALT$^{Ink4a/b}$, from the locus *Cdkn2a/b*, which is a potent cell cycle inhibitor. We hypothesize that the expression of pALT$^{Ink4a/b}$ maintains cells in a quiescent instead of a senescence state, thus preventing cells gero-conversion. In this work we evaluated the effect of heterologous expression of pALT$^{Ink4a/b}$ in mouse embryonic fibroblasts in a model of DNA damage-induced senescence.

Transcription analysis of endothelial cells treated with tumor soluble factors of cell line ZR-7530

Andrei Ivan Favela Orozco[1,2], Alberto Jose Cabrera Quintero1,2, José Luis Ventura Gallegos[1], Beatriz Mora Ramiro[2], Mayte Cervantes Badillo[1,2,3], Minerva Janini Mejía Rangel[2], Martin Arturo Gallardo Vera[1,2], Juan Pablo Aragón Hernández[4], Jorge Román Audifred Salomón[4], Alejandro Zentella Dehesa[1,2,3]

[1]Programa Institucional de Cáncer de Mama y Departamento de Medicina Genómica y Toxicología Ambiental, Instituto de Investigaciones Biomédicas de la UNAM.
[2]Unidad de Bioquímica, Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán".
[3]Centro de Cáncer, Centro Médico ABC Observatorio.
[4]División de Ginecología y Obstetricia del Hospital General Dr. Manuel Gea González.
Address: Circuito Mario de la Cueva s/n, Coyoacán, Ciudad Universitaria, 04510 Ciudad de México, CDMX. Email: azentell@iibiomedicas.unam.mx

Abstract

Presently, recurrence and metastasis are the main causes of death among adult patients with breast cancer. In the metastatic process, tumor cells must overcome physical and immunological barriers, to survive and colonize distant target sites. In this sites an decuate microenvironment fosters tumors cells through a complex interaction between the humoral component of the primary tumor, acting in an endocrine manner that affect normal cells at the distant site.

Among the different lineage of normal cells exposed to the tumor humoral component, the cells that cover the inner part of the vascular system. Those endotelial cells are permanent sentinel on all components of the blood stream, which makes them a checkpoint for the transmigration of the tumor metastatic cells.

Our line of reserch has studied, the response of primary cultures of human endothelial cells to the tumor humoral component (tumor soluble factors), secreted in vitro by the breast cancer cell line ZR-75-30. The expression profile at 3 hours post-stimulus, showed a dominance of the NFkB pathway. This expression pattern is in contrasts with the fact that among the tumor soluble factors (TSF) there are components capable of activating other signaling pathways such as VEGF or IL6.

Therefore in the present work, we explored the transcriptional profile of endothelial cells post-exposure to TSF of ZR-75-30 cells at short times (15, 30, 60 min). Interestingly, in response to the TSF, we observed changes in the phosphorylation state of STAT3´s Tyr705 and expression of the response genes of this pathway.

The importance of cell context: Ski and SnoN regulatory mechanisms in hepatocytes

Diana G. Ríos-López, Cassandre Caligaris , Genaro Vázquez-Victorio, Marcela Sosa-Garrocho, Alvaro Marín-Hernández, Marina Macías-Silva

Instituto de Fisiología Celular, Universidad Nacional Autónoma de México. Ciudad de México, C.P. 04510. MEXICO. e-mail: drios@email.ifc.unam.mx

Ski and SnoN proteins function as transcriptional co-repressors in the TGF-β pathway. They regulate cell proliferation and differentiation, and their aberrant expression results in altered TGF-β signalling, malignant transformation, and alterations in cell proliferation. We carried out a comparative characterization of the endogenous Ski and SnoN protein regulation by TGF-β, cell adhesion disruption and actin-cytoskeleton rearrangements between normal and transformed hepatocytes; we also analyzed Ski and SnoN protein stability, subcellular localization, and how their protein levels impact the TGF-β/Smad-driven gene transcription.

We observed that Ski and SnoN protein levels are lower in normal hepatocytes than in hepatoma cells. They exhibit a very short half-life and a nuclear/cytoplasmic distribution in normal hepatocytes opposed to a high stability and re- stricted nuclear localization in hepatoma cells. Interestingly, while normal cells exhibit a transient TGF-β- induced gene expression, the hepatoma cells are characterized by a strong and sustained TGF-β-induced gene ex- pression. A novel finding is that Ski and SnoN stability is differentially regulated by cell adhesion and cytoskeleton rearrangements in the normal hepatocytes. The inhibition of protein turnover down-regulated both Ski and SnoN co-repressors impacting the kinetic of expression of TGF-β-target genes.

Normal regulatory mechanisms controlling Ski and SnoN stability, subcellular localization and expression are altered in hepatocarcinoma cells.

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Wnt pathway-related Extracellular Antagonists expression in osteoblast-like cells

Alma Y. Parra-Torres, Juana Enríquez and Rafael Velázquez-Cruz
Laboratorio de Genómica del Metabolismo Óseo, Instituto Nacional de Medicina Genómica (INMEGEN). Periférico Sur No. 4809, Col. Arenal Tepepan, Delegación Tlalpan. México, D.F. C.P. 14610, Phone. +52 (55) 5350-1900.
rvelazquez@inmegen.gob.mx *yoli_379@hotmail.com*

Osteoblasts are specialized cells derived from mesenchymal progenitors and responsible for bone formation and recovery of loss of bone mass by the activity of bone-resorbing osteoclasts. Osteoblast activities result from a series of molecular and biochemical events modulated by an integrated cascade of regulatory factors that are supporting early activities in cell differentiation and differential gene expression during osteoblast maturation. Recent studies have shown important roles of Wnt/β-catenin or canonical pathway in the regulation of proliferation, cell fate and differentiation of osteoblasts. Additionally, Wnt signaling pathway is finely regulated at extracellular level by diverse factors, including Secreted Frizzled-related proteins (SFRPs), Dickkopf proteins (DKK), Wnt inhibitory factor (WIF1), and SOST, a factor secreted by osteocytes. The extracellular Wnt antagonists play an essential role by regulating osteoblasts function and bone formation processes. The purpose of this study was to investigate the expression profile of extracellular Wnt signaling-related antagonists during a particular period of osteoblast development, early and late differentiation. During this period, the change of active Wnt signaling to its attenuation could increase the expression of antagonists in osteoblast cultures. To determine the expression pattern of extracellular antagonist genes during these stages in hFOB 1.19, MG-63 and Saos-2 cells lines, we used RT-qPCR and ALP activity assays. The relationship between ALP activity and expression of RUNX2 and OSX confirmed the presence of an active osteoblast differentiation during the late stage of cell culture (15-21 days), in the three osteoblast-like cells lines. Although the expression of extracellular Wnt antagonists varied between cells lines, the increase of expression was consistent with the late differentiation in all cells lines tested. The overexpression of AXIN2 during the late differentiation indicated an attenuation of the Wnt/β-catenin signaling pathway at this stage. This finding suggests that the combination of all extracellular antagonists contributes to the balance between early and late differentiation stages. This work presents a widespread profile of changes in gene expression during maturation of osteoblast-like cell lines, commonly used in osteoblast differentiation experiments, and offers a new window for research of Wnt signaling in bone metabolism. Additional studies are needed to elucidate their role in the osteoblast maturation.

Pseudomonas chlororaphis as a new model for shikimic acid production.

Alma Alva; Fabián Moreno; Francísco Bolívar; Adelfo Escalante.
Instituto de Biotecnología, UNAM. Av. Universidad #2001, Col. Chamilpa C.P.62210 Cuernavaca, Morelos. Tel. +52 (777) 329-1601.
email: adelfo@ibt.unam.mx

Shikimic acid (SA) is an intermediate of the shikimate pathway which it´s naturally found in plants, fungi, bacteria and some parasites. SA is used as a precursor for different pharmaceutical products such as antivirals, antitumorals, aromatic aminoacids, antiobiotics etc, because of that, there are different strategies to obtain these compound being plants and bacteria the principal source.

The main microorganism studied for the overproduction of SA has been *E. coli* but, after many years of study, the maximum titer obtained has been 87 g/L. Considering these, we propose a *Pseudomonas chlororaphis* ATCC 9446 as potential microorganism for the overproduction of SA, some intermediates upstream of this pathway such as DHQ and DHS and other aromatics derivate of chorismate.

P. chlororaphis is an innocuous and ubiquitous microorganism of rice, soy and tomatoes roots which has been used recently for the production of some chorismate derivate such as phenazines because of its characteristics like a carbohydrate transport independent to PTS, the ability to metabolize different carbon sources like glycerol, sucrose, manosa and even some toxic compounds such as octanol and its resistance to some physicochemical conditions.

Until now, there are any reports for the production of shikimic acid or any intermediate of the shikimate pathway studied in *Pseudomonas* spp. considering these situation, we developed our strategy considering the methodologies followed in *E. coli* and for the overproduction of phenazines in *Pseudomonas*.

Our first approach was to inactivate by a double recombination methodology the enzyme shikimate kinase in order to avoid the flux from AS to shikimate-3-phosphate, after that, we inactivated one of the two pyruvate kinase (codified by *pykA* and *ttuE*) obtained the strains A2 (*aroK*$^{-}$ *pykA*$^{-}$*)* and A3 (*arok*$^{-}$ *ttuE*$^{-}$) to evaluate if there are any difference between them like in *E. coli* and finally, we constructed a plasmid (pUCP24A5) which codifies for the genes of *E. coli aroB, tktA, aroG*^{fbr}*, aroE* and *aroD.*

We evaluated all the strains in a modified King medium changing the carbon source (glucose or glycerol) in 1 L bioreactors founding that glucose is a better carbon source than glycerol achieving a mix of DHQ (1.16 g/L), DHS (0.24 g/L) and shikimate (0.01 g/L) in the strain A25/pUCP24A5. The differences observed between A2 and A3 with the plasmid suggest that the pyruvate kinase codified by these *Pseudomonas* have a different activity and/or regulation.

Now, we are focus in optimize our expression system for the overexpression of the genes and try to figure out which is the bottle neck or if there are any other pathway which can compete with the shikimate pathway for the carbon, likewise, we are probing a mixture of glucose and glycerol to figure out if these medium could increase our results.

Bacterial biodegradation of endocrine disruptors di-(2-ethylhexyl) phthalate and di-isononyl phthalate

Marco Antonio Pereyra Camacho, Víctor Emmanuel Balderas Hernández, Antonio De León Rodríguez*. Instituto Potosino de Investigación Científica y Tecnológica A.C. Departamento de Biología Molecular, Camino a la Presa San José 2055, Col. Lomas 4ª secc., C.P. 78216, San Luis Potosí, SLP.
*aleonr@me.com, aleonr@ipicyt.edu.mx, Tel. 01 (444) 834 2000 Ext. 2044

Phthalic acid esters (PAEs) or phthalates are a group of xenobiotic compounds widely used as plasticizers to improve mechanical properties in plastic products[1]. Special attention has been paid to PAEs because several studies have shown that they have toxicity to humans and animals due to xeno-estrogenic and endocrine-disrupting effects[2]. Since the rates of photolysis and chemical hydrolysis of phthalate esters are very slow, metabolic breakdown of PAEs by microorganisms is considered to be one of the main routes of environmental degradation of these pollutants[3]. The aim of this study is to identify bacteria capable of degrading di- (2-ethylhexyl) phthalate (DEHP) and di-isononyl phthalate (DINP) from 2 bacterial collections (50 isolates; tolerant to heavy metals and tolerant to salinity). The experiments were carried out in Mineral Salt Medium (MSM) and the conditions for the degradation of 500 mg L^{-1} of PAEs were pH 6.8, 30°C, and an agitation rate of 180 rpm by 4 days. Microorganisms that used DEHP and DINP as the sole carbon and energy source were selected using the enrichment-culture technique. The results showed that 11 isolates were capable to grow in DEHP and DINP as the only source of carbon and energy. The genera identified were *Bacillus* sp., *Serratia* sp., *Acinetobacter* sp., *Brevibacterium* sp. Metabolites of DEHP and DINP degradation were analyzed by gas chromatography–mass spectrometry (GC-MS). This study could provide an alternative to solve the problems of phthalate pollution.

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Keywords: xenobiotic, plasticizers, biodegradation, bacteria, DEHP, DINP

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Aspergillus sydowii, a halophile fungus with potentials to degrade lignocellulose, polycyclic aromatic hydrocarbons and pharmaceuticals: a transcriptomic and biochemical view

Ramón Alberto Batista-García[a,c*], Hubert Cabana[c], Heidy Peidro-Guzmán[a], Yordanis Pérez-Llano[a,b], Deborah González Abradelo[a], María del Rayo Sánchez-Carbente[b], Jorge Luis Folch-Mallol[b], Verónica Lira-Ruan[a]
[a]Centro de Investigación en Dinámica Celular - Universidad Autónoma del Estado de Morelos
[b]Centro de Investigación en Biotecnología - Universidad Autónoma del Estado de Morelos
[b]Université de Sherbrooke, Sherbrooke, Canada

Av. Universidad 1001, Col. Chamilpa. CP 62209. Telf 7773297000 ext 3276
*rabg@uaem.mx

Extremophile fungi have been claimed as powerful systems to conduct studies related with xenobiotic degradation. Halophile fungi are defined as those fungi with optimal growth under hypersaline conditions. *A. sydowii* was isolated from a sugarcane bagasse fermentation under 2 M NaCl. This fungus was classified as halophile since it shown its optimal growth between 0.5 and 1.5 M NaCl. *A. sydowii* has great potentialities to colonize different lignocellulosic substrates as sugarcane bagasse, sawdust corn, wheat straw and agave fibers. It produced cellulases, xylanases, peroxidases and lacasses on these substrates, enzymes with a high halostability. The transcriptomic profile of this fungus on wheat straw under hypersaline conditions (1 and 2 M NaCl) revealed a set of genes differentially expressed with a direct relation with halophilia and lignocellulose deconstruction. On other hand, this fungus has excellent properties to remove xenobiotic compounds from liquid media, as well as, from wastewaters obtained from biorefineries. Different studies have been conducted to demonstrate its potentialities to degrade, adsorb and remove approximately fifteen polycyclic aromatic hydrocarbons and ten pharmaceutical from liquid media. During xenobiotic degradation this fungus produced extracellular enzymes involved in biodegradation, such as lacasses, peroxidases and esterases. Moreover, its transcriptomic profile revealed more than 200 genes differentially expressed when the fungus is grown on benzo-a-pyrene and phenanthrene under hypersaline conditions. Halophile fungi are absolutely underexploited in relation with their biotechnological applications in xenobiotic degradation. Thus, more studies are required for a completely understanding about the molecular biology and biochemistry of these fungi. This work brings the first approach to study the potentialities of *A. sydowii* since molecular and biochemistry point of view. There is not information related with global studies (omics approaches) to understand the molecular biology and gene expression dynamic of halophilic fungi in presence of xenobiotics. This is the first attempt to get information from transcriptomic profiles of halophilic fungi growing in hypersaline conditions supplemented with xenobiotics.

Rational design of chimeric endolysins with application in aquaculture

César Salvador Cardona--Félix*, Lina Angélica Zermeño--Cervantes, María Zulema Juárez Cortés

* Instituto Politécnico Nacional (IPN), Av. IPN s/n, Col. Playa Palo de Sta. Rita, La Paz, B.C.S., México. C.P. 23096. 6121234658 ext. 81565
cscardonafe@conacyt.mx

Endolysins are enzymes encoded by bacteriophages at the end of their replication cycle to degrade the peptidoglycan of the bacterial host, resulting in cell lysis. In Gram-- positive bacteria, due to the absence of an outer membrane, endolysins can access the peptidoglycan and destroy microorganisms when applied externally. However, the expansion of endolysins as antibacterials against Gram--negative pathogens is hindered by the outer membrane. Therefore, the rational design of chimeric endolysins to generate antimicrobials with potentiated activity and a lower probability of generating said resistance has been proposed as an alternative. In this work, we carried out the rational design of a new chimeric endolysin (so--called LysVPMS1--PCNP). We use as a model the structural analysis of the wild--type endolysin (so--called LysVPMS1) from the phage VPMS1. These enzyme, exerts lytic activity on sensitized cells of the genus *Vibrio*. To potentiate the bacteriolytic activity, we modified the N--terminal domain of LysVPMS1 by fusing a polycationic peptide (PCNP). We corroborated the design performing *in silico* analysis by molecular coupling. Models also showed that the new protein is thermodynamically stable and the peptide is exposed. The recombinant protein was obtained and the muralytic activity was tested against permeabilized cells of *V. parahaemolyticus*. Chimeric endolysin showed 5.8 times more activity than the wild type enzyme. Our results shown that rational design of a chimeric protein can be carried out enhancing muralytic activity since this new endolysin obtains greater lytic capacity on permeabilized cells in less time.

An orthogonal system for beta-branching in polyketide biosynthesis

Pablo Cruz-Morales[1,2] & Jay D Keasling[1]
pcruzmorales@lbnl.gov
Joint Bioenergy Institute[1]. Centro de Biotecnología FEMSA Instituto Tecnológico y de Estudios superiores de Monterrey, Campus Monterrey[2].

Keywords: Synthetic biology, chemical biology, Polyketide synthases, biofuels and bioproducts

Polyketides are an abundant and diverse class of naturally occurring organic molecules that include many important pharmaceuticals. These molecules are synthesized in a iterative mechanism driven by modular megasynthases known as Polyketide Synthases (PKS). PKSs are formed by homologous domains that condense Acyl-CoA precursors to form ketones that are further reduced to form carbon chains of diverse length with aldehyde, alcohol, olefin or fully reduced functionalities, leading to an outstanding structural diversity in their products.

Given their iterative mechanism and modularity, large efforts are in place to engineer them with the aim of fully controlling length, branching and reduction with the promise of creating the ultimate biocatalyst for organic synthesis. Most efforts have focused in the exchange and selective inactivation of domains from well characterized PKS, however such efforts have yield little success potentially due to little control (and knowledge) over complex interdomain interactions.

Trans acting PKS domains are single-domain enzymes that interact with the PKSs megasynthases in multiple events during the product assembly. Among trans acting domains, Beta-branching enzymes are remarkable as they can produce ethyl, methyl, vinyl, carboxyl and chlorethyl functionalities in beta positions.

Given the potential of this system for PKS engineering we used a combination of phylogenomics and synthetic biology to select for bioparts to generate a beta branching component that can be orthogonally used to modify extant PKSs without affecting inter modular dynamics in the assembly line.

After phylogenomic analyses we selected the biosynthetic pathway for Bongkrekik acid as a source of bioparts, and used them to design, construct and test a system to produce 5C branched organic molecules of industrial relevance. I will present the approach, logic behind the design, and the results of experimental proof of concept.

Identification and Classification of Single Nucleotide Polymorphisms as Biomarkers associated with Dementia through Data Mining and Machine Learning

Erick Cuevas Fernández, Carlos Moncada Vázquez, Joel Sánchez Mendez, Montserrat Loredo Guillen, Heriberto Manuel Rivera*.

*Laboratorio de Tecnología de Proteínas de Membrana, Facultad de Medicina, Universidad Autónoma del Estado de Morelos (UAEM). Posgrado en ciencias Bioquímicas, Universidad Nacional Autónoma de México (UNAM). Calle Iztaccíhuatl esq. Leñeros s/n, col. Los Volcanes, Cuernavaca, Mor. CP 62350. 7772123774, erickcf@ibt.unam.mx, m2mrivera@uaem.mx; @ltpm_mx.

Abstract

Dementia is an acquired brain syndrome characterized by a decline from a previous level of cognitive functioning which it is usually chronic or progressive. It consists of deterioration of several higher cortical functions, such as memory, thinking, comprehension, calculation, learning, language and judgement. The cognitive impairment is not attributable to normal aging and significantly interferes with independence in the person's performance of daily living activities, more ever the people who suffer it and their familiar welfare and economical environment are affected. In Mexico it is estimated at 800,000 persons affected and is projected that it will almost three times at 2050, so the impact of this disease on the economic, social and health systems will be unprecedented. In the world, the costs of dementia are estimated at around 2 trillion dollars in 2030. The identification and classification of biomarkers, raises the possibility in a patient with mild cognitive impairment of circumscribe the origin of the deficit, or established reliable early diagnosis in asymptomatic stages for subsequent medical assistance. The human genome harbors all its information in 46 chromosomes, in which 3.2 billion base pairs are found, it is estimated that every 300 base pairs a single nucleotide polymorphism (SNP) is generated. There are about 1,803,563,957 variants in the SNP database, approximately 840,000 variants associated with a phenotype at ClinVar database, and 70,000 SNPs from genome-wide association studies. To handle this immense amount of data, it is necessary to use artificial intelligence (AI) tools. The ability and effectiveness of AI methods derive basically from the ability to identify and extract patterns to create models through complex collections of data, this computational capacity can be defined in different types of machine learning. The knowledge of patterns extracted from a complex database to make sense the interpretation of a biological phenomenon is carried out through a process of multistep data processing (selection, preprocessing, transformation, data mining, interpretation and evaluation). In this work we describe and classify SNPs as biomarker for different types of dementia: Alzheimer, vascular dementia, Lewy bodies disease, and frontotemporal dementia; as well as rare manifestations of dementia such as Creutzfeld-Jakob and AIDS dementia.

Evidence of the Red-Queen Hypothesis from Accelerated Rates of Evolution of Genes Involved in Biotic Interactions in *Pneumocystis*

Luis Delaye, Susana Ruiz-Ruiz, Enrique Calderon, Sonia Tarazona, Ana Conesa, Andrés Moya
CINVESTAV Irapuato
Km. 9.6 Libramiento Norte Carr. Irapuato-León 36824
Irapuato Gto. México
Tel: +52 (462) 6239669
E-mail: luis.delaye@cinvestav.mx

Abstract

Pneumocystis species are ascomycete fungi adapted to live inside the lungs ofmammals. These ascomycetes show extensive stenoxenism, meaning that each species of Pneumocystis infects a single species of host. Here, we study the effect exerted by natural selection on gene evolution in the genomes of three *Pneumocystis* species. We show that genes involved in host interaction evolve under positive selection. In the first place, we found strong evidence of episodic diversifying selection in Major surface glycoproteins (Msg). These proteins are located on the surface of *Pneumocystis* and are used for host attachment and probably for immune system evasion. Consistent with their function as antigens, most sites under diversifying selection in Msg code for residues with large relative surface accessibility areas. We also found evidence of positive selection in part of the cell machinery used to export Msg to the cell surface. Specifically, we found that genes participating in glycosylphosphatidylinositol (GPI) biosynthesis show an increased rate of nonsynonymous substitutions (dN) versus synonymous substitutions (dS). GPI is a molecule synthesized in the endoplasmic reticulum that is used to anchor proteins to membranes. We interpret the aforementioned findings as evidence of selective pressure exerted by the host immune system on *Pneumocystis* species, shaping the evolution of Msg and several proteins involved in GPI biosynthesis. We suggest that genome evolution in *Pneumocystis* is well described by the Red-Queen hypothesis whereby genes relevant for biotic interactions show accelerated rates of evolution

Modeling the structure-function relationship in biological systems with machine learning

Fernando Fontove, Gabriel Del Río
Instituto de Fisiología Celular, UNAM. Cd. Universitaria 04510 Cd. De México.
gdelrio@ifc.unam.mx.

BACKGROUND

The relationship between protein structure and function represents a fundamental quest where perception of reality (function) is related to physical measurements of it (structure). Since protein representations of structure and function are different (e.g., atomic 3D coordinates versus catalytic activity), current methods aimed to tackle this problem include two steps: i) choosing a representation of the protein structure and ii) transformation of that representation to map it to the function representation. In this work we evaluated the possibility to identify a single representation of proteins that may be used for both structure and function learning.

METHODS Construction of an adjacency matrix from a protein 3D structure was derived by a distance criterion, where two amino acids are considered to be in contact if their distance is less than a cutoff value D. RCC[1] are derived from the contact graph by identifying maximal cliques of size 3 to 6 amino acids and divided into 26 categories depending on the type of interaction the clique forms.

CATH protein structure classification was divided in 3 categories: C, CA and CAT. GO protein function classification for each PDB entry was derived by cross-referencing with the Uniprot database, resulting in multiple chains from the same PDB entry assigned to multiple functions; the protein function was divided in three groups: Cellular Localization (C), Molecular Function (F) and Biological Process (P). For every protein in the PDB its RCC was calculated varying the distance threshold D (3-15 Å). Auto-weka[2] was used to select the best performing algorithm and optimized parameters. Protein-Protein interactions were predicted using the 3DID database and Negatome 2.

RESULTS We identified that a random forest algorithm and RCC calculated without the lateral chains and D=7 rendered the best models to learn protein structural and GO functional classification. Comparing the performance of our models with state of the art CAFA1[2][3] on Fmax score (C=.46, F=.59, P=.37) shows C is on par (.44), F underperforms (.24) and P performs slightly better (.41). Though the CAFA results use a different data set and their approach uses the amino acid sequence. Additionally, if the protein space is restricted to those with a single chain the performance improves significantly (C=.58, F=.48, P=.54).

CONCLUSIONS

The RCC provides a single representation useful for predicting both structure and function that has results comparable to state of the art tools.

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Modeling the regulatory network of lymphopoiesis

Luis Mendoza
Instituto de Investigaciones Biomédicas, UNAM
Circuito Exterior s/n Ciudad Universitaria CP04510, CDMX, México.
lmendoza@biomedicas.unam.mx
Tel: +52(55) 56229210

The hematopoietic system is one of the most extensively studied systems in mammals, resulting in a detailed knowledge of the pattern of production of blood cells. Furthermore, due to the number and nature of diseases caused by the malfunction of hematopoiesis, there is a large interest in knowing the molecular mechanisms that control the differentiation of the cellular elements of blood.

To understand the molecular mechanisms controlling the production and differentiation of cells during hematopoiesis, there has been a steady creation of mathematical and computational models that incorporate a large body of published experimental data. My research group is pioneer in the inference and analysis of regulatory networks of different modules of hematopoiesis, specifically on T, B, and NK cells lymphopoiesis. Currently, we are working on an integrative model that aims at uniting the three aforementioned modules into a single regulatory network model.

I will present the latest advances on the inference and analysis of the regulatory network controlling lymphopoiesis in mammals. Currently, the network consist of 100 nodes, representing molecules and molecular complexes, plus the set of regulatory interactions among them. For its analysis the network has been converted into a continuous dynamical system with the use of the SQUAD methodology. Results so far show that the model is able to describe the main aspects of T, B and NK lymphopoiesis, starting from the Common Lymphoid Progenitor, when the system is subjected to simulated external signaling.

The cellular mechanisms of lifespan extension by metformin

Michelle Crisely Munguía Figueroa, Cei Abreu Goodger and Alexander de Luna Fors
Unidad de Genómica Avanzada (Langebio), Centro de Investigación y de Estudios Avanzados del IPN
Irapuato, Gto. México, CP 36824, +52 (462) 166 3000 ext. 3069 alexander.deluna@cinvestav.mx

The antidiabetic drug metformin extends the lifespan of mice[1], yeast[2] and nematodes[3]. While the mechanisms by which it exerts its effects are just starting to be characterized, it is still unclear which are the relevant genes to its lifespan extension effect and what are the underlying systems-level mechanisms. Here, we perform a large-scale functional genomics assay to identify genes, processes, and pathways that participate in metformin longevity extension in the budding yeast *Saccharomyces cerevisiae*. Specifically, we measured the stationary-phase survival of single-gene knockout strains grown on metformin-supplemented medium and compare the lifespan extension effects of the knockout strains compared to the wild type. We focus our study in single-gene knockout yeast strains for 1478 genes with an orthologue in the human genome; genes showing gene-environment interactions defined as changes in the phenotypic effect of the knockout strain to metformin are mediators of metformin lifespan extension. To address a second level of regulation, we will use RNA-Seq to measure transcriptomic metformin response. Our study will provide the first large-scale genomic view of how metformin modulates cellular mechanisms leading to longevity.

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Effect of antibiotics in the ecological dynamic of microbial communities.

Daniela Reyes González , Rafael Peña Miller, José Utrilla Carreri and Ayari Fuentes Hernández. Laboratorio de biología de sistemas y biología sintética, Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México, Av. Universidad s/n, Chamilpa, 62210 Cuernavaca, Morelos. ayarifh@ccg.unam.mx

Microbial communities are complex organizations usually composed of a big taxonomic diversity capable of realizing a huge amount of functions, some studies have shown that the microorganisms inside each community are able to communicate with each other in different ways, creating different types of relations that determine the robustness and the dynamic of the community.

Besides the taxonomic and functional diversity, the environment composition plays an important role in the ecology of each community, the absence of some metabolites in the environment allows the presence of strains with specific metabolism like amino acid auxotrophies.

The presence of auxotrophic phenotypes within a community forces its members to exchange specific metabolites that allow their coexistence (cooperation). Interactions between members can change over time giving differences in the dynamic and changing the robustness of it by the increase or decrease of cooperation. Environmental modifications are another way to change cooperation. Antibiotics in the environment can induce changes in the populations composition (Liu et al 2015).

Using eight different *Escherichia coli* amino acid auxotrophy strains we build synthetic consortia with obligated metabolic interactions and determine the different dynamics of strain pairs using the growth of the co-cultures in a determined time, with different initial proportions of each phenotype, we determine the proportions with higher growth and according to the dynamic we change the environment using a bacteriostatic antibiotic at sub-lethal concentrations as a way of modifying the interactions. We also explore how different environmental conditions like adding certain amino acid to the media, can change the susceptibility profile of the consortia changing the stability of the interaction.

Identification and selection of genetic biomarkers for atherosclerosis

Javier Álvarez Arroyo[1], María Montserrat Loredo Guillén[1], Joel Sánchez Méndez[1], Erick Cuevas Fernández[1] and Heriberto Manuel Rivera[1].
Laboratorio de Tecnología de Proteínas de Membrana, Facultad de Medicina. Universidad Autónoma del Estado de Morelos Calle Iztaccíhuatl esq. Leñeros s/n, Col. Los Volcanes, 62350, Cuernavaca, Morelos Tel: 777 3297000 ext 3500 y 3493
m2mrivera@uaem.mx. Facebook:LTPMmx ;Twitter:LTPM_mx

Abstract

According to the WHO (World Health Organization) in 2016, 15.2 million deaths were caused by atherosclerosis-related diseases like; myocardial infarction and/or stroke.

Such pathologies rank among the primary causes of death worldwide. Unfortunately, in Mexico the trend prevails, where the ischemic diseases amass 13.4% of all the annual deaths. The AHA (American Heart Association) estimated that the annual cost of aforesaid diseases would reach $818 billion USD in 2030. Although the physiopathological process remains unclear, the developing of the atheromatous plaque in the arteries, formed by a cluster of LDL (Low- Density Lipoproteins) and involving the participation of many cell types (platelets, macrophages, neutrophils, lymphocytes, inflammatory cells, etc.,) play preponderant roles in the installation of the atherosclerotic disease. There are several nosological factors that contribute to the development of atherosclerosis, such as, Diabetes Mellitus, Hypertension, Obesity, Hypertriglyceridemia, Insulin Resistance and elevated LDL levels. The progression of the disease takes from a few years to several decades and is unnoticeable, causing sudden thrombotic events. Currently, the clinical detection of this pathology takes place on late stages. Therefore, new alternatives such as genetic risk prediction trough risk score in an early stage of atherosclerosis based on SNPs identification are necessary.

The principal aim of this work is the identification and selection of genetic biomarkers for atherosclerosis in early stages. In order to do this, a selection, preprocessing, transformation, data mining, interpretation, and evaluation of big data through a genomic approach was used. As a result, 25,829 atherosclerosis-related SNPs were identified, 159 of those SNPs are reported in a GWAS (Genome-Wide Association Study). Several of those SNPs are linked to the lipid metabolism and the transport of small molecules, taking part in the regulation of the atherosclerotic process, conceding a genetic-based risk prediction. Finally, we look forward to future clinical implementation in Mexican population.

Establishment of *ex vivo* and *in vivo* models for the study of bone remodeling

Brenda Ivette Iduarte-Frias, Liria M Fajardo-Yamamoto, Pierrick GJ Fournier, Patricia Juárez*.

Centro de Investigación Científica y de Educación Superior de Ensenada, Departamento de Innovación Biomédica. Carretera Ensenada-Tijuana No. 3918, Zona Playitas, C.P. 22860, Ensenada, B.C. México ***Corresponding author email** – pjuarez@cicese.mx

The skeletal system is a highly dynamic tissue thanks to a process called bone remodeling that occurs throughout our life. Bone remodeling allows the bone to maintain its integrity and fulfill its functions through balanced cycles of resorption and formation.

However, bone remodeling can become imbalanced, generating pathologies that

compromise bone functions locally or systemically. Three types of bone cells are associated with this process: the osteoclasts, responsible for bone resorption; the osteoblasts that produce and mineralize the bone matrix, and the osteocytes, involved in mechanotransduction activities.

Osteoporosis is characterized by a decreased bone mineral density that compromises bone strength, increases the risk of fractures, physically debilitating, and leading to a drastic decrease in quality of life. Worldwide, an osteoporotic fracture occurs every 3 seconds, 1 in 3 women over the age of 50 will experience osteoporotic fractures, as will 1 in 5 men aged over 50. To understand the mechanisms involved in bone remodeling, whether under normal or pathological conditions, improve actual therapies, and continue the search of new potential therapeutic agents, it is necessary to develop models that recreate the bone microenvironment.

Therefore, we worked on the establishment of three models. 1) As an osteoclastogenesis model, the bone marrow cells from BALB/C mice were cultured in the presence of M-CSF and RANKL. Nine days after induction, we validated the model by performing TRAP staining to quantify osteoclasts and used qRT-PCR to evaluate the expression of osteoclastic genes. 2) Osteoblasts were differentiated using isolated cells from neonatal mouse calvarias. Mouse calvarias were digested using collagenase, the solated cells (osteoblast precursors and primary osteoblasts) were cultured for 21 days in the presence of ascorbic acid and β-glycerophosphate. Alkaline Phosphatase and Alizarin Red S staining were performed to validate the model. 3) Osteoporosis was modeled *in vivo* using ovariectomy. Ovariectomy was performed on females C57BL/6 of 8 and 16 weeks, one month later the mice were euthanized, and the bones were collected to evaluate bone loss by histology, or to measure the expression of genes associated with bone remodeling (Opg, Alpl, Runx2, Rankl, Acp5, Ctsk, MMP13).

We found the right conditions to successfully recreate the bone microenvironment in *ex*

vivo and *in vivo* models. Our results showed that we can use the implemented models to study bone formation and bone resorption. Unbalanced bone remodeling represents a severe public health problem. Worldwide, more than 200 million patients are affected by osteoporosis; we can use these models to reach a better understanding of this disease and assess potential therapeutic agents.

Profibrotic Role of Matrix Metalloproteinase-28 and Potential Diagnostic Biomarker for Idiopathic Pulmonary Fibrosis

Mariel Maldonado[1], Ivette Buendía-Roldán[1], Alfonso Salgado-Aguayo[1], Iliana Herrera[1], Moisés Selman[1], Annie Pardo[2]*.

[1]Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Mexico city, Mexico. [2]Facultad de Ciencias, Universidad Nacional Autónoma de México, Mexico city, Mexico. *Facultad de Ciencias, UNAM. Ciudad Universitaria, Ciudad de México 04510. Tel:(55) 56233822. apardos@unam.mx

Idiopathic Pulmonary Fibrosis (IPF) is a progressive and generally lethal epithelialfibroblastic disease whose diagnosis is based on the identification of the usual interstitial pneumonia (UIP) pattern either by high resolution computed tomography and/or histology. However, a similar pattern can be observed in other fibrotic lung disorders with better prognosis and different treatment, so precise diagnosis remains challenging. Studies on diagnostic biomarkers are scanty.

Matrix Metalloproteinases (MMPs) are a family of zinc-dependent enzymes that play important roles in the extracellular milieu, as well as in the cytoplasm and even in the cellular nucleus. Some MMPs are upregulated in IPF and participate in its pathogenesis and/or progression. We have shown that one of them, MMP28, is expressed by the alveolar epithelial cells localizing inside the nuclei (Images were selected for this July AJRCMB cover). Results from silencing and overexpressing the protein *in vitro* and from the murine model of fibrosis with wildtype and deficient mice showed that MMP28 induces a profibrotic phenotype. Our aim is to evaluate MMP28 as a biomarker for distinguishing IPF from fibrotic non-IPF patients, as well as suggest mechanisms through which this enzyme exerts its effects.

Serum concentration was determined by ELISA in two independent cohorts of patients, derivation cohort included 82 IPF and 69 non-IPF patients, as well as 36 healthy controls; while validation cohort enclosed 42 IPF, 41 non-IPF, and 11 controls.

Our results show that MMP28 is significantly upregulated in serum from IPF patients versus non-IPF and age-matched controls ($p<0.001$). The AUC of the derivation cohort was 0.718 (95%CI, 0.635-0.800). With a cutoff point of 4.5 ng/mL, OR was 5.3 (95%CI, 2.55–11.46), and sensitivity and specificity of 70.7% and 69% respectively. The AUC of the validation cohort was 0.690 (95%CI, 0.581-0.798), OR 4.5 (95%CI, 1.76–12.04), and sensitivity and specificity of 69.6% and 66.7%.

The concentration of this enzyme is higher in IPF patients versus non-IPF patients with UIP pattern. In addition, we are studying whether MMP28 effects may be exerted via other proteases such as MMP2, MMP9 or MMP14.

Our findings suggest that MMP28 is significantly upregulated in the serum of IPF patients and may be a useful biomarker to improve diagnostic certainty.

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Aging and absence of *Zmpste24* protects premature aging mice from developing bleomycin-induced pulmonary fibrosis through the overexpression of antifibrotic microRNAs.

Jazmín Calyeca[1], J Raúl Olmos [2], Rogelio Jasso [2], Miguel Gaxiola [2], Quetzali Rivera [1], Yalbi Bálderas [2], Vilma Maldonado [3], Moisés Selman [2] and Annie Pardo [1].

[1]Facultad de Ciencias, Universidad Nacional Autónoma de México, Mexico City, Mexico; [2] Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Mexico City, Mexico; 3 Instituto Nacional de Medicina Genómica, Mexico City, Mexico. E-MAIL:calyecajaz@gmail.com, Tel. 56-22-48-00 Ext 84076.

Idiopathic Pulmonary Fibrosis is a progressive and lethal disease of unknown etiology. Strong evidence indicates that aging is a driving force of the disease and the incidence and prevalence of IPF increases remarkably with age. Notwithstanding, the mechanisms linking IPF to aging are still uncertain. There are scanty works using old mice models of lung fibrosis, the main problem lies in the significant practical difficulties associated with the generation of aged mice, including time and cost. There is growing interest to identify experimental models of accelerated aging. Recently, it was shown that *Zmpste24* deficient mice displayed accelerated aging. Zmpste24 is a zinc metalloproteinase responsible for the final cleavage step of nuclear envelop prelamin A, a critical step for its maturation process. May be useful to understand some aging-associated mechanisms that may contribute to the development of fibrosis.

To clarify the role of aging in the development of lung fibrosis and to unveil whether *Zmpste24* deficient mice could be an appropriate aging-model for this purpose, we examined their fibrotic response to bleomycin-induced lung damage.

Unexpectedly, we found that old but not young *Zmpste24* deficient mice seem to be protected to the development of bleomycin-induced lung fibrosis. The attenuation of the fibrotic response was confirmed by a significant decrease in lung hydroxyproline content. Global gene expression analysis revealed an increased expression of several antifibrotic microRNAs including miR23a, miR27a, miR29a, miR145a and miR29b-1 in bleomycin damaged lungs of *Zmpste24* deficient mice, which was validated by qPCR. As expected, several targets of these microRNAs, including *Tgfβ*, *collagen* 1 and 3, and some *Mmp´s* genes were decreased.

Conclusions: Our results suggest that accelerated aging induced by the absence of *Zmpste24*, result in attenuated fibrotic response the expression of miRNAs that target extracellular matrix targets mRNAs.

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Restoration of circadian levels of NAD^+ reduces physiopathology caused by a high-fat diet

M. en C. Quetzalcoatl Escalante Covarrubias, Dra. Lorena Aguilar Arnal
Instituto de Investigaciones Biomédicas, UNAM
Ciudad Universitaria 04510, Coyoacán, CDMX
562-28938, loreaguilararnal@iibiomedicas.unam.mx

The circadian clock is an endogenous system that regulates the oscillations of various biological variables in approximately 24 hr cycles linked to external signals such as light [1]. At the molecular level, the circadian clock is made up of autoregulatory feedback loops of transcription and translation. The transcriptional activators are the proteins BMAL1 and CLOCK, which heterodimerize, and bind to consensus sequences called E-boxes to promote the transcription of clock-controlled genes, including the negative regulators of the feedback loop, *Period* (*Per1-3*) and Cryptochrome (*Cry1-2*). PER and CRY proteins can inhibit BMAL1-CLOCK activity, which decreases the transcription of circadian genes [2] including Nicotinamide phosphoribosyltransferase, the rate-limiting enzyme in the NAD^+ salvage pathway, which drives oscillations in NAD^+ metabolite itself [3]. NAD^+ levels regulate certain metabolic pathways via modulation of oxidation-reduction reactions and the enzymatic activity of SIRT1, a NAD^+-dependent histone deacetylase. Importantly, SIRT1 also targets many energy metabolism regulatory proteins for deacetylation, hereby impacting their function. [4].

Interestingly, NAD^+ levels drop in the liver of high fat diet (HFD)-fed mice, which show pathologies related to metabolic syndrome [5]. Therefore, this work intends to elucidate how restoration of circadian rhythms on NAD^+ levels impact pathologies associated with obesity and metabolic syndrome. To this end, a cohort of 60 C57BL/6 mice was fed a normal diet and a HFD diet for 10 weeks. Half of the group fed a HFD was pharmacologically treated for circadian restoration of NAD^+ levels fduring three weeks, starting atweek 7 of the feeding paradigm.

So far, our results indicate that circadian restoration of NAD^+ in HFD-fed mice successfully decreased serum glucose and triglyceride levels, improved insulin and glucose tolerance test results, decreased lipid accumulation in the liver, and recovered circadian transcription of several clock genes.

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Omega-3 fatty acids in diabetes

Ricardo Mejía Zepeda, Ramiro Perusquía García, Diana A. Martiñon Ramírez, Raymundo Coate Camacho, Uriel A. Vicenteño Monroy, Karla Avendaño Briseño, Jorge Escutia Martínez, María Fernanda Campos Guzmán, and María del Consuelo Figueroa García.

Lab 4. UBIMED. Facultad de Estudios Superiores Iztacala, UNAM.
Av. De los Barrios No. 1. Los Reyes Iztacala, Tlalnepantla, Estado de México, México. C.P. 54090.
Tel. 5623-1138. rmejia@unam.mx

Diabetes Mellitus (DM) is a health problem widely extended all over the world. According to the information provided by the Atlas of the International Diabetes Federation (IDF 2017), there are around 425 million people with diabetes. Closely associated to the development of the diabetes and other diseases such as obesity, hypertension, and dyslipidemias, is the lipid metabolism. It is interesting that a group of essential fatty acids known as omega-3 (ω3), are reported to have beneficial effects against diabetes, although there is not a clear idea of the mechanism by which they might have effects. It is known that fatty acids fulfill multiple functions in cell biology; from structural buildings blocks of membranes and energy producers, to precursors of signaling molecules and gene expression regulators also. The essential polyunsaturated omega-3 fatty acids (ω3) may have a list of functions that still are under research. It is essential to learn more about the effects and function of ω3 in order to know their mechanism(s) of action and consequently, the appropriate way to used them safety. In this work we present summarized some of the results that we have obtained about the effect of omega 3 fatty acids on models of diabetes developed in rats. Although we have to analyzed the effect of diabetes and omega 3 fatty acids on the whole animal model, we have focused our interest on the physicochemical properties of biological membranes, and some processes that take place in them, especially in mitochondria.

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Defensin ϒ-Thionin from *Capsicum chinense* induces apoptosis in the humanbreast cancer cell line MCF-7 and regulate histone H3 epigenetic modifications

María T. Arceo-Martínez,[1*], Jaquelina J. Guzmán-Rodríguez[1], Zoraya Palomera-Sánchez[1], Alejandra Ochoa-Zarzosa [1], Joel E. López-Meza.[1]

(1) Centro Multidisciplinario de Estudios en Biotecnología, Universidad Michoacana de San Nicolás de Hidalgo. Km 9.5 Carretera Morelia-Zinapécuaro, La Palma. Tarímbaro, Michoacán. CP 58893, México. Tel/Fax: 01(443)295-8029. Autor para correspondencia: marithe.13_@hotmail.com

Cellular homeostasis is regulated by genetic and epigenetic mechanisms controlling cell proliferation and differentiation; dysregulation in these functions has been associated to cancer. Breast cancer is considered the first worldwide cause of cancer death in women. Antimicrobial peptides (AMPs) have showed cytotoxic activity against diverse cancer cells through different mechanisms; however, only the effects of animal AMPs have been well characterized. Therefore, the aim of this work was to determine the cytotoxic effect of the plant defensin ϒ-thionin on MCF-7 breast cancer cells and to characterize its mechanism of action. The cytotoxic effect of ϒ-thionin on MCF-7 cells was assessed by trypan blue exclusion assays. This AMP was cytotoxic in a concentration-dependent manner, with an IC_{50}=117.29 µg/ml, but viability of normal bovine mammary epithelial cells and peripheral blood mononuclear cells was unaffected. Additionally, this AMP induced apoptosis in MCFcells and did not affect the membrane integrity or cytosolic calcium release. Further,ϒ-thionin IC_{50} increased the expression of apoptosis related genes such as *caspase 7, 8, 9*, *Apaf-1* and *cytochrome c*. Importance of epigenetic modifications of histones have been recognized in all of the stages of many types of cancer. We characterize the epigenetic modifications H3K9ac, H3K9me2/3 and H3S10fos associated to cytotoxic activity of ϒ-thionin. This AMP increased H3K9ac after 24 h of treatment. Also, this AMP showed significant inhibitory activity against HDAC at 6 h of treatment. Further, the mRNA expression of deacetylases HDAC1/2/3 decreased after 24 h of treatment. Also, the level of H3K9me2 was increased while the expression of histone demethylase KDM4A and the α-estrogen receptor gene (Esr1) decreased at 24 h of treatment. Interestingly, the expression of a-estrogen receptor target gene cyclin D1 was diminished. Nevertheless, H3S10ph was unchanged in all of the times analyzed. Taken together, these results show that ϒ-thionin induces apoptosis in MCF-7 breast cancer cells and regulates changes in genetic expression, which could be associated with H3 epigenetic modifications. This is thefirst study of a plant AMP inducing cytotoxicity through epigenetic mechanisms in a breast cancer model.

CTCF-KDM4A complex correlates with histone modifications that negatively regulate the tumor suppressor *CHD5* gene expression in cancer cell lines.

Lissania Guerra-Calderas[1,2], Rodrigo González-Barrios[2], Carlos César Patiño[1,2], Nicolás Alcaraz[3], Marisol Salgado-Albarrán[1,2], Clementina Castro Hernández[2,4], and Ernesto Soto-Reyes[1].

[1].Natural Sciences Department, Universidad Autónoma Metropolitana (UAM), Mexico City 05300, Mexico. [2].Cancer Biomedical Research Unit, Instituto Nacional de Cancerología (INCan), Mexico City, Mexico. **3**.The Bioinformatics Centre, University of Copenhagen, Copenhagen, Denmark. [4].Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México (UNAM), Mexico City, Mexico.

Corresponding author: Soto-Reyes, E. UAM-C, Av. Vasco de Quiroga 4871, Col. Santa Fe, Del. Cuajimalpa de Morelos, 05348, CDMX. email. esotoreyes@correo.cua.uam.mx

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INSTITUTO NACIONAL DE CANCEROLOGÍA, UNIVERSIDAD AUTÓNOMA METROPOLITANA

The histone demethylase KDM4A is involved in H3K9me3 and H3K36me3 demethylation, which are epigenetic modifications associated with gene silencing and RNA Polymerase II elongation, respectively. *KDM4A* is abnormally expressed in cancer, affecting the expression of multiple targets, such as the *CHD5* gene. This enzyme localizes at the first intron of *CHD5*, and the dissociation of this protein increases *CHD5* gene expression. *In vitro* assays showed that KDM4A-mediated demethylation is enhanced by the presence of CTCF, suggesting that CTCF could increase its enzymatic activity *in vivo,* however the specific mechanism by which *CTCF* and *KDM4A* might be involved in the *CHD5* gene repression is poorly understood. Here, we show, that CTCF and KDM4A form a protein complex, which is recruited into the first intron of *CHD5*. This is related to a decrease in H3K36me3/2 histone marks and is associated with its transcriptional downregulation. Depletion of *CTCF* or *KDM4A* mediated by siRNAs, triggered the reactivation of *CHD5* expression, suggesting that both proteins are involved in the negative regulation of this gene. Furthermore, the knockout of *KDM4A* restored the *CHD5* expression and H3K36me3 and H3K36me2 histone marks.

Additionally, ChIP-seq and ChIP-Re-ChIP-seq assays demonstrated that CTCF and KDM4A coexist in 9 sites along the *CHD5* gene, in particular at the intronic regions. Overall, our findings support a novel mechanism of epigenetic gene regulation at the gene body that is independent of the promoter region.

CTCF regulates IL6 expression in breast cancer cells

Gustavo Ulises Martinez-Ruiz[1, 2], Guillermo Aquino-Jarquin[1], Ricardo Valle-Rios[1], [2], Jorge Melendez-Zajgla[3], Miguel Angel Quevedo-Castillo[1, 2].
[1]Hospital Infantil de México Federico Gómez, CDMX, México
[2]Division de Investigación, Facultad de Medicina, Universidad Nacional Autónoma de México, 04510, CDMX, México
[3]Instituto Nacional de Medicina Genómica, CDMX, México
Address: 162 Dr. Marquez, Cuauhtémoc, Mexico City, 06720
Phone: 52289917 ext. 2368
e-mail: gustavobambam@gmail.com

Abstract
In breast cancer, overexpression of IL6 gene is associated with worse clinical outcomes. Since both in vitro and in vivo models demonstrate that the autocrine expression of IL6 gene mediates the survival of breast cancer cells to anticancer drug-mediated cell death, the identification of molecular mechanisms explaining its transcriptional activation in cancer cells is urgently needed. On the other hand, CTCF is a multifunctional protein that regulates the tridimensional configuration of the human genome through its interaction with specific genomic sites. In this way, CTCF drives the transcriptional landscape. In breast cancer, CTCF has been associated with drug resistance for its ability to regulate transcriptional activation from key genes. Here, we analyzed whether CTCF is able to regulate IL6 transcription in breast cancer cells. CTCF had repressive role on IL6 expression at both mRNA and protein levels from both gain- and loss-of-function assays of CTCF that were performed in breast cancer cell lines. To delve in the regulatory mechanism of CTCF on the transcription of the IL6 gene, we identified two putative binding sites of CTCF in the IL6 promoter by using MWM. We confirmed that those binding sites repressed IL6 gene expression by site-directed mutagenesis and promoter-gene-reporter assays. Due to MCF7 and MDA-MB-231 cell lines showed lower and higher IL6 expression levels, respectively, we performed ChIP assays using antibodies against CTCF to analyze whether CTCF binding to the IL6 promoter is associated with those expression levels. As expected, we determined that CTCF bound to the promoter of the IL6 gene in MCF7 cells but not in MDA-MB-231 cells. To determine the biological weight of this CTCF-IL6 axis in antitumor drug resistance, a tamoxifen-resistant cell line was generated from MCF7 cells. As hoped, this cell line showed higher expression levels of IL6 than its parental MCF7 cells. Importantly, we demonstrated that CTCF was unable to interact with the IL6 promoter in the tamoxifen-resistant cell line explaining its highest expression levels of IL6 gene.
In general, CTCF seems to mediate malignancy in breast cancer by regulating gene expression through its association with DNA-regulatory sequences such as the promoter of the IL6 gene.

Methylation pattern of the human *ATP2A3* gene promoter in gastric and colon cancer cell lines

Angel Zarain-Herzberg[1]*, Iván Meneses-Morales[1], Eduardo Izquierdo-Torres[1], Lucía Flores-Peredo[1], and Gabriela Rodríguez[1]
[1]Department of Biochemistry, School of Medicine, National Autonomous University of Mexico. Mexico City, 04510, México. Tel.: +52 (55) 5623-2258, Fax: +52 (55) 5616-2419, E-mail: zarain@unam.mx

Gastrointestinal cancer constitutes a leading cause of death in many countries. The knowledge about the role of calcium-regulated pathways in cancer cell growth and differentiation could serve for the development of new therapeutic approaches to diminish its mortality. SERCA pump proteins modulate cytosolic Ca^{2+} concentration, regulating various cellular processes including tumor cell growth. *ATP2A3* gene silencing was suggested as an early step in tumorigenesis and decreased SERCA3 expression found in several tumor tissues samples, even at the adenoma stage. *ATP2A3* gene transcriptional regulation has been studied in gastric and colon cancer cell lines, but there is still a lack of understanding about the epigenetic processes regulating its transcription.

In this work, we report the methylation status of the CpG island spanning from position −320 to +409 of the *ATP2A3* gene. We found an increase in the methylation status of the -235 to -135 bp promoter region that matches with a diminished transcriptional activity of the promoter and correlates with the transcriptional code of histones associated to this region demonstrated by ChIP assays. Butyrate and trichostatin A treatments induced de-methylation of this regulatory CpG-rich region, inducing SERCA3 re-expression and cell differentiation, increased apoptosis and decreased cell viability of the KATO-III gastric carcinoma cell line. In agreement with the above findings, *in silico* SERCA3 expression in gastric and colon tumors, as well as overall survival results show that high SERCA3 expression could serve as a favorable prognostic marker for colon and gastric cancer patients.

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Candida glabrata cis-acting element Sil2126 negatively regulates the expression of adhesin-encoding genes through chromatin loop formation

Eunice López-Fuentes, Grecia Hernández-Hernández, Leonardo Castanedo, Guadalupe Gutiérrez-Escobedo, Katarzyna Oktaba, Alejandro De las Peñas, and Irene Castaño*
*IPICYT. Instituto Potosino de Investigación Científica y Tecnológica. División de Biología Molecular. Camino a la Presa San José 2055, San Luis Potosí, SLP, 78216, Mexico, (444) 8342000, icastano@ipicyt.edu.mx

Adherence is an important virulence factor in several pathogens. A higher virulence has been correlated with increased adherence and with an expansion of adhesin-encoding genes. In *Candida glabrata*, the adherence to host cells is mainly mediated by *EPA* (Epithelial Adhesin) genes. Most of *EPA* genes are located in subtelomeric regions regulated by subtelomeric silencing, which depends on Rap1, Ku proteins and the SIR complex. Epa1 mediates most of the adherence to epithelial cells *in vitro*. The *EPA1* gene forms a cluster with *EPA2* and *EPA3* in the right telomere of chromosome E (E_{-R}). Between *EPA3* and the telomere, there is a *cis*-acting element, the protosilencer Sil2126, which requires its own telomere context for its activity. Sil2126 can silence a reporter gene 32 kb away from the telomere (a region normally where there is no silencing). Our results showed that there are *cis*-acting elements located in the *EPA2-EPA3* intergenic region that are required for Sil2126 activity, which perhaps are responsible for the Sil2126 telomere-specificity. The 5' end of Sil2126 contains putative binding sites for Rap1 and Abf1; we found that these proteins bind to Sil2126 in its original position and also when it is placed at -32 kb (Sil@-32kb). Rap1 and Abf1 also bind to other regions in the E_{-R} subtelomeric region. In addition, we detected that Sil@-32kb can recruit Sir3, suggesting the propagation of the silencing up to that distance. We used 3C (Chromosome conformation capture) assays to measure crosslinking frequencies between Sil@-32kb and Sil2126 in its original position across the E_{-R} subtelomeric region. These assays revealed that Sil@-32kb interacts with the *EPA1-EPA2* intergenic region forming a loop that results in the repression of *EPA1*. And Sil2126 interacts with the *EPA2-EPA3* intergenic region. We propose that the mechanism of action of Sil2126 is through the recruitment of silencing proteins to form a superstructure involving different interactions between *cis*-acting elements and different proteins.

Genetic analysis of aging factor SWR1 in *Saccharomyces* and *Drosophila*

Judith Ulloa Calzonzin[1,2], Katarzyna Oktaba Sosin[2], Alexander De Luna Fors[1].
[1]Unidad de Genómica Avanzada (Langebio) y [2]Departamento de Ingeniería Genética, Centro de Investigación y de Estudios Avanzados del IPN
Irapuato, Gto. México, CP 36824, +52 (462) 166 3000 ext. 3069 teodora.ulloa@cinvestav.mx, k.oktaba@cinvestav.mx alexander.deluna@cinvestav.mx

Aging is an accumulation of damage across time that causes cellular and functional decline in a living organism. This phenomenon is determined by different genes and pathways, many of which are conserved across different species. We previously reported that SWR1, a complex involved in histone exchange and chromatin modification is a novel aging factor in the budding yeast *Saccharomyces cerevisiae* (Garay *et al*. PLOS Genetics, 2014). Specifically, inactivation of SWR1 extends the chronological lifespan of yeast. Here, we report a detailed genetic analysis of different subunits of SWR1 and the related NuA4 complex in yeast. We also show that longevity by inactivation of SWR1 is conserved across different genetic backgrounds in *S. cerevisiae*. Moreover, we generated double mutants of *Δswr1* with 303 genes related to aging and longevity, which has allowed us to describe its genetic-interaction profiles and the pathways that are needed to extend lifespan by deletion of this gene. SWR1 recognizes phosphorylation of H2A and drives exchange of H2A for the histone variant H2A.Z. This function is conserved from yeast to human, however, in animals, SWR1 is part of the larger TIP60 complex. Therefore, we are currently investigating whether inactivation of TIP60 subunits results in extended lifespan in *Drosophila melanogaster*. Our genetic analysis of SWR1 sheds light on the roles of chromatin regulation in aging and longevity across species.

Mixing synthetic ecology and metagenomics towards mine waste bioremediation

Luis D. Alcaraz[*1], Miguel Romero[1], Diana Gallego[1], Jazmín Blaz[1], Arely Lechuga[1,2], Hugo Barajas[1], José Martínez Rodríguez[3], Francisco Molina-Freaner[3], and Rocío Cruz-Ortega[2].
[1] Laboratorio de Genómica Ambiental. Departamento de Biología Celular. Facultad de Ciencias. [2] Departamento de Ecología Funcional y 3 Departamento de Ecología Funcional del Instituto de Ecología. Universidad Nacional Autónoma de México 04510 Cd. Mx. México
*lalcaraz@ciencias.unam.mx http://genomica.fciencias.unam.mx

In our laboratory, we make use of classical microbe isolation, mesocosm experiments, microbiome analysis, and shotgun metagenomics to study plant-microbe interactions. We are a multidisciplinary team involving multiple backgrounds from ecology, plant physiology, bioinformatics, and molecular microbiology aiming for long-term bioremediation strategies to cope with the sizeable unconfined mine tailings spreading in Mexico. A fantastic natural phytostabilization experiment is underway in an abandoned copper mine tailing in Nacozari, Sonora, where pioneering local plants were able to establish in the wasteland. Plant colonization helps to prevent open mine tailings environmental and human health impacts by decreasing erosion and aeolian transport of heavy metals into food-chains or water sources. We are studying the microbial impact on plant establishment and health within wastelands. For the moment, we identified 422,381 OTUs (55 bacteria and 3 archaea phyla) in four in situ plant species; found differences related to nutrient and heavy metal contents. The metagenomic diversity showed vast dominance by Actinobacteria and Proteobacteria and over-representation of ion efflux transporters of the RND superfamily. Finally, we assembled a synthetic community from the culturable bacteria from Nacozari rhizospheres with 244 strains co-inoculated in an experimental evolution mesocosm selecting for two traits: heavy metal resistance and early plant colonization. The synthetic community was described by shotgun metagenomics, and we are looking for plant-growth promotion and plant establishing capabilities of the synthetic community under heavy metal stress.

Strategy to identification of mutations in resistant *Mycobacterium tuberculosis* isolates in Jalisco Mexico

Guadalupe Gutierrez-García[1], Martin López-Rodríguez[2], Natividad Ayala-Chavira[2], Blanca Torres-Mendoza[3], Eduardo Vázquez-Valls[4] and Ikuri Álvarez-Maya[1]
[1]Medical and Pharmaceutical Biotechnology Unit, Center for Research and Assistance in Technology and Design of the State of Jalisco (CIATEJ), Av. Normalistas No. 800, Col. Colinas de la Normal, C.P. 44270 Guadalajara, Jalisco, [2] Laboratory of State of Public Health, Ministry of Health Jalisco. [3] Department of Philosophical, Methodological and Instrumental Disciplines, University Center of Health Sciences, University of Guadalajara. Sierra Mojada 950, Colonia Independencia 44340, Guadalajara, Jalisco. [4] Human Immunodeficiency and Retrovirus Laboratory, Western Biomedical Research Center, Mexican Institute of Social Security, Guadalajara, JAL, Mexico Sierra Mojada 800, Colonia Independencia 44340, Guadalajara, Jalisco. E-mail address: ikuri.alvarez@gmail.com

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis,* is considered one of the main causes of death worldwide. The diagnostic methods commonly used in Health Centers of developing countries, is one of the limitations for TB control. Thus, efforts have been increased to develop diagnostic methods and technologies effective, low cost and portable.

The aim of the study was to analyze the complete genomic sequence of *Mycobacterim tuberculosis* from clinical isolates.

For DNA isolation the bacteria were heated to boiling, and then the DNA was purified by the CTAB method. The genomic library of purified DNA was constructed using the Nextera XT (Illumina) kit and the next generation sequencing was performed on the MiSeq platform of Illumina.

The complete sequence of *Mycobacterium tuberculosis* was assembled and the alignment was performed. The assembly of sample A and B had quality to be analyzed, achieving more than 97% similarity with the reference. Sample C had a low assembly quality, due to the low number of high quality reads for the assembly. The mapping of sample B indicates an optimal coverage mapping ≥30x (46.29x); the mapping of sample A indicates a coverage lower than the limit suggested for the analysis ≥30x (28.58x); and for sample C, the coverage level is below the suggested minimum value for any mapping analysis (12.73x). For sample B the number of variants or detection of SNPs indicates a high quality of detection and reliability. While for sample A the number of variants or detection of SNPs indicates a threshold below the detection quality (depth of the 28.58x mappings), therefore, the number of reads would have to be increased to have a high reliability. The sample C the number of variants or detection of SNPs are not reliable, thus requires a greater number of reads to increase the depth.

A later analysis will allow the identification of point mutations in the genome, to compare with the literature and know if these regions have been reported in other strains.

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Characterization of a putative TCS implicated in the response to salinity stress in *Rhizobium etli* CFN42.

David Correa-Galeote, Susana Rodríguez, David Zamorano-Sánchez and Lourdes Girard.
correa@ccg.unam.mx.

Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México (UNAM), 62210, Cuernavaca, México. Phone number +52 1777 313 4152.

Two-component systems (TCS) are the prevalent signalling scheme in bacteria, they coordinate the response to environmental changes through modulating transcription of target genes. The prototypical TCS consist of a sensor histidine kinase (HK) and a response regulator (RR). The HK transfers a phosphate group to the RR and this modification affects its activity as an elicitor of an specific response. The OmpR family is the most abundant family of RRs, and includes well characterized transcriptional regulators such as *E. coli* OmpR and PhoB (important for osmoregulation and phosphate assimilation respectively). In *Rhizobium etli*, a soil dwelling bacteria that can form a symbiosis with the common bean *Phaseolus vulgaris*, 17 of its 68 RRs belongs to the OmpR family. Despite its importance for survival under harsh conditions in multiple organisms, *in R. etli* the OmpR/PhoB-like regulators remain poorly characterized. During its life cycle, *R. etli* is exposed to changes in osmotic pressure and salinity; however, this bacteria lacks a prototypical EnvZ/OmpR TCS that would elicit an adequate response to this stress.

In this work, we aimed to characterize the role of members of the OmpR family and their predicted cognate HKs, in the response of *R. etli* CE3 to salinity stress.

Our systematic evaluation of different *R. etli ompR* mutants, revealed that orf RHE_RS12325 is required for optimal growth in the presence of 100 mM NaCl. Interestingly, the absence of its putative cognate HK (RHE_RS12320) resulted in a moderate increase in tolerance to 100 mM NaCl. We also evaluated the capacity of these mutants to establish an effective symbiosis with *Phaseolus vulgaris* both in control condition and in the presence of NaCl. Additionally, we analysed the participation of the genes annotated as porins in the *R. etli* genome in the response to the osmotic stress and the dependence on RHE_RS12325 for its expression.

The results revealed that the RHE_RS12325 regulator is involved in the response to salinity stress modulating the expression of different porins. Interestingly, the absence of this regulator enhanced the capacity of *R. etli* to fix nitrogen in association with bean plants.

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Identification and characterization of a family of outer membrane proteins of *Helicobacter pylori*, which scavenges iron from human sources.

José de Jesús Olivares-Trejo[1]*, Marco Antonio González-López [2], Cristhian Sánchez-Cruz[1].
[1]*Posgrado en Ciencias Genómicas, Universidad Autónoma de la Ciudad de México, San Lorenzo 290, Col. Del Valle, C.P. 03100, Ciudad de México, México. *e-mail: olivarestrejo@yahoo.com. Tel: +525554886661 Ext. 15309.
[2] Cátedra CONACYT-Instituto Nacional de Cancerología. Av. San Fernando No. 22, Col. Sección 16, C.P. 14080. Ciudad de México, México.

Helicobacter pylori is a gram-negative spiral bacterial, it has been associated with peptic ulcers, gastritis, duodenitis and it is believed to be the causative agent of gastric cancer. The sources such as human lactoferrin, haem and haemoglobin can support the *H. pylori* growth. However, we do not yet fully understand how the process of iron acquisition occurs. We have evidences of a family of proteins that is expressed in membrane and has a high affinity for iron, because these proteins were characterized and we think that they can be regulated by the iron source. The last protein of the family was purified by haem-affinity chromatography and its capacity of binding haem was demonstrated. This protein was identified by mass spectrometry as FrpB3 (Putative iron-regulated outer membrane protein). The 3-D models of both proteins showed that they are structurally conserved and they have the typical barrel structure, which is inserted in membrane. Also FRAP and NPNL motifs were reveal they are necessary for Hb-binding. However, the data cannot explain how these proteins are regulated. To answer this question their respective expression was quantified by real time technique under different human iron sources. We observed that FrpB1 was overexpressed with haem, while FrpB2 was induced in presence of haem and also haemoglobin. In the case of FrpB3, it was overexpressed with haemoglobin. For this reason, we propose that *H. pylori* secretes proteins in order to withstand the extreme environment present in the stomach. Our overall results represent the effort to explain the importance of iron acquisition. Perhaps iron helps the bacterium to resist the acidic environment of the human stomach and this mechanism is vital for *H. pylori* during the infection process.

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Inhibition of *las* quorum sensing system is not enough to suppress virulence of *Pseudomonas aeruginosa*

Martín Paolo Soto-Aceves. Miguel Cocotl-Yañez. Israel Castillo-Juárez. Gloria Soberón-Chávez. Instituto de Investigaciones Biomédicas, UNAM / Apdo. Postal. 70-228, Ciudad de México 04510, México / 01(55)56229201 / martin.soto@iibiomedicas.unam.mx

Pseudomonas aeruginosa is a Gram negative opportunistic pathogen of plants and animals, its intrinsic resistance to many antibiotics make it one of the main cause of nosocomial infections leading to high mortality and morbidity rates. *P. aeruginosa* virulence is due to the variety of extracellular factors that it produces such as elastase, alkaline protease, rhamnolipids, pyocianin, among others. The synthesis of these factors is regulated by the quorum sensing system (QSS), which is a process involving cell-density-dependent accumulation of signal molecules that together with a regulator protein modulate the expression of specific genes. This bacterial species has three QSS; two of them based on the detection of N-acyl-homoserine lactones (*las* and *rhl* systems), and one based on the detection of 2-alkyl-4(1H)-quinolones (PQS system); these systems are correlated and regulated hierarchically being *las* the system on the top. Consequently many studies are targeting *las* system in order to block the entire QS response and suppress virulence. However, some strains deficient in *las* system still produce virulence factors suggesting that this system may not be the best target.

In this work, antisense RNAs and mutants were constructed to silence and block *lasR* and *rhlR,* genes encoding the regulator proteins of the *las* and *rhl* systems respectively, in order to determine which of these two systems can be the best target to inhibit the QSS and reduce the virulence factors production.

Using the artificial RNA technology, it was possible to obtain inhibition only against LasR. In this case we obtained 38% inhibition, assayed with a transcriptional fusion, and a similar rate in the apparently decrease of LasR detected by western blot. However this decrease has no impact in the production of elastase or quinolones, which are regulated by LasR.

Furthermore, *lasR* and *rhlR* single mutants and a double mutant *lasR-rhlR* strain were constructed. Virulence factors production and virulence in mice were evaluated. Results showed that in absence of LasR virulence factors production and virulence were reduced but not suppressed, suggesting that even though *las* system regulate the others systems, it is not the only regulator that can turn them on. The *rhlR* mutation impairs the synthesis of elastase and abolishes pyocianin production but the virulence in mice was also only reduced, not suppressed. However, in the double mutant the production of the virulence factors tested were completely abolished and also the virulence on mice. Together these results show that is necessary to target both *las* and *rhl* systems in order to suppress the global virulence of *P. aeruginosa.*

STRATEGIES OF *Avibacterium paragallinarum* TO SUCCESSFUL COLONIZE HOST TISSUES

[1]María Elena Cobos Justo, [2]Brenda Romero Hernández, [2]Fernando Pérez Cortés [1]Patricia Sánchez Alonso, [3]Erasmo Negrete Abascal, [4]Víctor Pérez Márquez, [1]Candelario Vázquez Cruz. [1]Centro de Investigaciones en Ciencias Microbiológicas. [2]Licenciatura en Biotecnología, Instituto de Ciencias. Benemérita Universidad Autónoma de Puebla. Edificio IC11, 24 Sur y Av. San Claudio. C.U., Col San Manuel, CP. 72570. Puebla, Pue. Tel: 2222295500, Ext. 2536. [3]Carrera de Biología. Facultad de Estudios Superiores Iztacala. UNAM. Avenida de los Barrios no. 1. Los Reyes Iztacala, 54090 Edo. de México. [4]BIOVETSA Tehuacán Puebla México. Email:ecobacilos@yahoo.com

Infectious coryza, a disease of the upper respiratory tract of birds is produced by *Avibacterium paragalinarum* (AVPG). The bacterial culture of AVPG is difficult because bacteria requires NAD, serum from animal blood, low oxygen pressure and unknown nutrient substances, and finally bacteria dies by infrequent reseeds. It is surprising and unexpected that a fastidious bacterium could be so persistent in the environment to produce epidemic cases of infectios coryza around the world. A possible explanation to this behavior is a very close metabolic relationship between bacteria and host tissues. An approach to understand the exigent metabolism of AVPG was made with genomic explorations. To pass unnoticed the bacterium shows a low metabolic activity, it depends to capture NAD and iron from host cells. AVPG lacks nicotinamide phosphoribosyl transferase but have transporters to import exogenous NAD. This bacterium produces siderophore aerobactin, hemin transport system and iron transporter regulated at low-pH. AVPG 2015 strain contains 29 genes to build cell walls, but CL strain lacks 4 genes from this set, this difference render at least two different antigenic polysaccharides. Some polysaccharides are similar in composition between bacteria and host cells, but some animal sugars are metabolized by bacterial enzymes. The bacterial binding is carried out by fimbriae F17-like and other type IV-like. All these components seem to play a role *in vivo* biofilm, like consortium; from fifteen strains an only one contains a large set of genes related with quorum sensing. This model is agreeing with metagenomics reported by other authors.

Phytochemical scrutiny and evaluation of the biological activity of wood Extracts

Malinali Ambriz-Ortiz[1], Josué Altamirano-Hernández[2], Crisanto Velázquez-Becerra[1].
[1]Facultad de Ingeniería en Tecnología de la Madera, Universidad Michoacana de San Nicolás de Hidalgo.
[2] Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo. 58040.
Teléfono: 01 443 4640511. Correo electrónico: malinaliamente@hotmail.com.

Introduction. Tree plant biomass is considered a valuable source of natural and chemical resources capable of preventing and minimizing suffering. An alternative in the treatment of diverse affections is the use of their extractables, since they present cytotoxic and antioxidant activity due to their phenolic composition. "For example" paclitaxel an organic alkaloid isolated from *Taxus brevifolia* sold commercially as cytotoxic used in different types of cancer. For this reason, the tree bark of the *Pinus* genre obtained by using a technique popularly known as "pica" in Michoacán in the extraction of resin, which has no commercial use, was analyzed. On the other hand, “palo dulce” a medicinal tree was analyzed also endemic to attend genitourinary infections and were used in these adjuvant extracts in the delay of the complications of multiple chronic-degenerative and infectious diseases.

Objective. Evaluate the biological activity of wood extractables.

Materials and methods. The hexanic, acetonic, tetrahydrofuranolic, methanolic and aqueous extraction of *Eysenhardtia polystachya (Ort.) Sarg.,* and *Pinus pseudostrobus, Pinus leiophyla* and *Pinus montezumae* were extracted. For in vivo tests, *Artemia salina* used in toxicological preliminary studies was used in the evaluation of plant compounds with anticancer potential, was evaluated the lethal dose 50, likewise the nematode *Caenorhabditis elegans* N2 (WT) to was induced oxidative stress with H_2O_2 to evaluate the protective and antioxidant capacity in biological organisms by the calculation of the LD50, the antimicrobial and antifungal effect of the extracts was also analyzed, through of the inhibition halo in the human opportunistic bacteria: *Ralstonia picketti*, *Campylobacter jejuni*, *Kocuria palustris* and *Micrococcus luteus*, and by the plate growth sensitivity technique in wood rot saprophyte fungi *Trametes versicolor* and *Gloeophyllum traveum*.

Results. The evaluation of toxicity in *A. salina* showed that the administration of the extractables obtained with methanol, acetone and hexane increased the mortality levels, in another way in *C. Elegans* the methanolic extracts of *E. Polistachya* and *Pinus* decreased the death values when was administered. With respect to the antimicrobial activity, it was observed that the methanolic, acetonic and tetrahydrofuranolic dosage in bacteria had greater effect in the inhibition halo, the first dose mentioned had a greater effect, it is used in traditional medicine for cure infections.

Conclusions. The extracts of *Pinus* and *E. polistachya* evaluated have antioxidant activity *in vivo*, also antimicrobial, so it can be a good candidate to prevent the oxidative stress present in chronic degenerative diseases.

Characterization of the interaction of the antimicrobial peptides Pin2 and Pin2 [GVG] using lipid membrane model systems

Brandt Bertrand, Sathishkumar Munusamy, Gerardo Corzo, Iván Arenas Sosa, Carlos Muñoz-Garay*

Instituto de Ciencias Físicas, Universidad Autónoma Nacional de México (ICF-UNAM). Avenida Universidad 2001, Chamilpa, 62210 Cuernavaca, Morelos México.

Carlos Muñoz-Garay. Instituto de Ciencias Físicas, Universidad Autónoma Nacional de México (ICF-UNAM). Avenida Universidad 2001, Chamilpa, 62210 Cuernavaca, Morelos México. Phone (777) 329 1802. E-mail address: cgaray.icf.unam@gmail.com

Introduction: The antimicrobial peptide (AMP) Pandinin 2 or Pin2 was first isolated and characterized from the African scorpion *Pandinus imperator* (Corzo *et al*. 2001). Pin2 is a 2.6 kDa cationic peptide with high activity against a wide range of gram-positive and gram-negative bacteria. However, its hemolytic activity hampers its clinical application. The Pin2 [GVG] mutant has potential for pharmaceutical use since it shows high antibacterial activity but is less hemolytic. Up to date the mechanism by which Pin2 or Pin2 [GVG] disrupt the host membrane has not been demonstrated, thus more knowledge on their molecular interactions with bilayer membranes is necessary. The aim of this study is to characterize the interaction of Pin2 and Pin2 [GVG] using different lipid models in order to better understand the factors that govern their antibiotic and hemolytic activity.

Materials and methods: The biophysical methods to be used are as follows: 1- *Liposome leakage experiments*. 2- *Langmuir isotherm experiments and Brewster angle analysis.* 3- *Light scattering analysis.* 4-*Infrared and Raman spectroscopy* and 5- *Atomic Force Microscopy*.

Results of calcein leakage experiments

a) **b)**

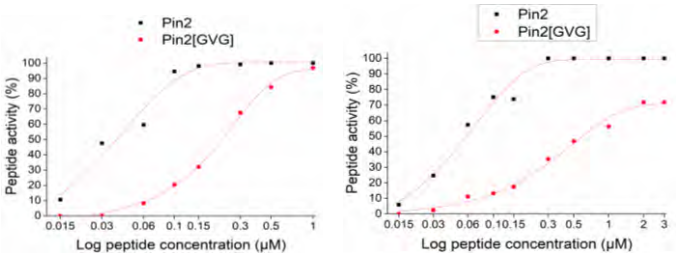

Table 1. Comparison of Pin2 and Pin2 [GVG] activity towards bacterial and erythrocyte LUV liposome models

LUV system	Peptide	Max activity and peptide concentration	EC_{50}
POPC:POPG (80:20)	Pin2	100 % at 0.15 μM	0.91
	Pin2[GVG]	97 % at 1 μM	1.01
POPC:PSM:Chol:POPE (34:33:25:8)	Pin2	100 % at 0.3 μM	0.29
	Pin2[GVG]	63.5% at 1 μM	3.34

Figure 1. Dose-response of Pin2 and Pin2 [GVG] in liposomes leakage. The plots show activity *vs*. the log of molar concentration: **a**) Liposomes made up of pure POPC:POPG and **b**) POPC:PSM:Chol:POPE (3433:25:8).

Conclusions: Pin2 was more active than Pin2 [GVG] in both systems (where the POPC:POPG and POPC:PSM:Chol:POPE systems represented bacterial and erythrocyte cells). This correlated well with reports of native cells *In vivo* experiments. The other biophysical experiments are currently being undertaken.

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FIG1-dependent cell death during cell cycle arrest in yeast.

Vladimir Juarez A.[1], Gabriel Del Rio[1]*

Biochemistry and Structural Biology Department, Instituto de Fisiología Celular,
Universidad Nacional Autónoma de México, México DF, México.
*Presenting author:vjuarez@email.ifc.unam.mx

Iztli Peptide-1 (IP-1) is the first antimicrobial peptide capable of selectively killing yeast cells during cell cycle arrest. In such stage, cells are resistant against current antibiotics; hence, understanding the mechanism of action of IP-1 may unravel new targets for a new class of antibiotics. From the screening of 3 744 null mutant yeast strains we identified that the null mutant of *FIG1* gene protected against IP-1 through a mechanism that does not involve the prevention of the cell cycle arrest. *FIG1* is a kinase required for the efficient pheromone-mediated mating process: it is involved in the low affinity Ca2+ influx system. Yet, null mutants of genes relevant to the calcium influx activity of Fig1p did not affect cell cycle arrest nor protected against IP-1 toxicity, indicating that the role of *FIG1* in IP-1-induced cell death is not related to its activity on calcium influx.

Knocking out the *FIG1* gene in yeast cells made them resistant to the IP-1 toxicity in a cell-cycle arrest mediated by the pheromone or other mechanisms. On the other hand, we identified the protein interactions of IP-1 in yeast cells, using cellular extracts in which IP-1 was adhered to a column. We identified that IP-1 interacts with Kar5p and that the null mutant of KAR5 protects against the cell death induced by IP-1 during a cell cycle arrest mediated by the pheromone or other mechanisms.

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Moringa oleifera seed prolamin fraction and its inhibitory capacity on the angiotensin converting enzyme-I.

Jesús Ricardo Pérez Velázquez, Mónica Andrea Valdez Solana, Miguel Aguilera Ortiz, Jorge Armando Meza Velázquez y Erick Sierra Campos.
Facultad de Ciencias Químicas de la Universidad Juárez del Estado de Durango, campus Gómez palacio.
Corresponding: Av. Articulo 123 S/N, Fraccionamiento Filadelfia C.P. 35010 ericksier@gmail.com

Most of the physiological activities of proteins are performed by sequences of peptides encrypted in the parent protein and have been shown various activities affecting, the digestive, immune, nervous and cardiovascular systems. However, these peptides only become active when they are cleaved during hydrolysis using proteolytic enzymes from animal, bacteria or plants being this latters the most used. In our previous study, the potential use of *Moringa oleifera* as a clotting agent of different types of milk (whole, skim, and soy milk) was investigated. *M. oleifera* seed extract showed high milk-clotting activity demonstrating its potential use in cheese making. In order to characterize the product from this hydrolysis, this work focused on the extraction of the protease present in the seeds and characterize the peptides generated by its use in the casein hydrolysis, the peptides obtained were evaluated for its inhibitory activity on the Angiotensin converting enzyme-I (ACE-I) to prove an antihypertensive effect. For the identification of the protease we obtained four soluble protein fractions from the seeds of *M. oleifera* according to its solubility with different solvents: albumins (aqueous), globulins (ionic buffer), prolamins (alcoholic buffer) and glutelins (basic buffers). Fractions were separated by SDS-PAGE to characterize the protein patterns. For the albumin fraction the proteins with apparent molecular weight of 60, 27, 22 and 20 kDa were observed. With the globulins the apparent proteins of ~70, 60, 55, 50, 35, 32, 28, 18, and 17 kDa were visualized. Under the conditions used in the assays, only one protein of ~28 kDa was resolved in the prolamin fraction, whereas in the glutelin fraction two proteins of ~29 and 18 kDa were resolved. In order to determine which fraction contained the proteolytic activity is found, casein was used to be hydrolyzed by the fractions during 60 min at 60° C. The highest degree hydrolysis of casein was found in the Prolamin fraction of 69% at 60 min and with the other fractions were 38% at 60 min. For rapid determination of the secondary structure and folding properties of the protein of the prolamin fraction we employed circular dichroism. For the ACE-I inhibitory activity of the hydrolysates of prolamin fraction we employed the method proposed by Shanguang in 2012, this method is based on the reaction of hippuric acid with *p*-dimethylaminobenzaldehyde in the presence of quinoline, acetate, and acetic anhydride. The red orange formation product in the reaction was determined at 478 nm. The results showed that the hydrolysates of casein using the fraction of prolamin presented a better ACE-I inhibitory activity. Finally, this study showed that hydrolysates of seed prolamins proteins had a nutraceutical potential.

Lupresan, a new drug that prevents or reverts the formation of lipidic particles that trigger a lupus mouse model

Carlos Wong Baeza; Claudia Albany Reséndiz Mora; Luis Alfonso Lamas García, Sandra Vela Patiño, Irene Nevárez Lechuga, Juan Carlos Santiago Hernández; Carlos Wong Ramírez; María Isabel Baeza Ramírez. Departamento de Bioquímica, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional. Prolongación de Carpio y Plan de Ayala s/n. C.p. 11340. Ciudad de México, México. Tel. 57296000 ext. 62321.
charlywong@icloud.com

The lipidic particles are lipid associations different from the bilayer that are transient, so they are not immunogenic. But if they are stabilized by some drugs such as chlorpromazine, they become immunogenic and induce anti-lipidic particle antibodies, which are found in patients with Systemic Lupus Erythematosus and are the ones that trigger a disease similar to Lupus in mice. Chloroquine, a drug used in the treatment of malaria, has been widely used to treat patients with Lupus; however, the mechanism of action by which it works in the treatment of Lupus is not known with certainty. In our research group, we have shown that chloroquine prevents the formation of lipidic particles or reverts the formed, so it could be its mechanism in the treatment of Lupus. On the other hand, we have also shown that the polyamine spermidine is more effective than chloroquine in preventing the formation of lipidic particles or in reverting the formed ones. Probably because this polyamine better stabilizes the lipid bilayer, because it has 3 positive charges in its structure at physiological pH, unlike the chloroquine that only has two. However, although polyamines participate in many biological functions, they cannot be used as drugs because when they have been administered in humans, they produce hematuria, proteinuria, renal failure and nephrotoxicity. In addition, in laboratory animals, they produce seizures, coma and death. So, in this work we design and synthesize Lupresan, an analogue of chloroquine with three positive charges at physiological pH. Lupresan was more effective in reverting or preventing the formation of lipidic particles than chloroquine or spermidine, and as a result, it decreased auto-antibodies titers and healed the malar rash in mice with lupus to a greater extent than chloroquine.

Highly purified extracellular vesicles from Fetal Bovine Serum reveal a mutually exclusive two-set of microRNAs

Filiberto A. Bautista-Moreno, Mariana Flores-Torres, O. Omar Moreno-Proo, S. Eréndira Avendaño-Vázquez, C. Fabián Flores-Jasso.

Instituto Nacional de Medicina Genómica
C.P. 14610, Phone +52 (55) 5350-1900 ext. 1138
Correspondence: cfflores@inmegen.gob.mx

Exosomes are microvesicles that range from 30–100 nm approximately and can mediate cargo transfer into a wide variety of recipient cells. For this reason exosomes have emerged as strong vehicles for small molecules and drug delivery. It was reported recently that microRNAs contained in extracellular vesicles from Fetal Bovine Serum (FBS) could transfer into cell cultures. In their work, Wei et al. separated vesicular from non-vesicular fractions by ultracentrifugation and concluded that the fraction containing extracellular vesicles (EVs) could mediate microRNAs transfer into cell cultures, potentially biasing the results of small RNA detection; RNA classes from FBS were detected by deep sequencing after FBS fractionation by ultracentrifugation. However, it is widely known that exosome pellets resulting from ultra-celtrifugation are often contaminated with non-vesicular proteins (such as argonaute), leaving open to the possibility that the identity for some RNAs, or their abundance, may be misrepresented. In this work, we present a procedure to obtain a

highly purified fraction of EVs from FBS that begins by size exclusion chromatography followed by pelletization via VN96 peptide, in order to characterize the RNAs present in exosomes, as compared with the RNAs from non-vesicular fractions from FBS.

We show that our method is easily scalable and removes a large amount of serum albumin, the most abundant protein component of FBS. We identified the RNA classes contained in six different brands of FBS and also characterized the fraction containing EVs, and the non-EVs fraction separately. By using our two-step purification we find that indeed there are a set of RNAs that are mutually exclusive between fractions and yet another group that it is apparently shared between them.

The differences between the EVs and whole FBS classes of RNA might be important when similar results are found in others biofluids that are used —or proposed as biomarkers, due to some of the RNAs present in the non-vesicular fraction could potentially interfere with diagnosis.

Multi-protein regulation of intracellular pH during human sperm capacitation

Arturo Matamoros-Volante&Claudia L. Treviño*
Departamento de Genética del Desarrollo y Fisiología Molecular, Institutode Biotecnología, Universidad Nacional Autónoma de México, 62250, Cuernavaca, Morelos, México.*ctrevino@ibt.unam.mx, tel. 7773291611.

To acquire fertilizing capabilities, mammalian sperm cells must undergone a series of maturation processes known as capacitation inthe female reproductive tract. Most of thebiochemical and morphological changes during capacitation,and the signaling pathways that control them, are pH-dependent, nevertheless, there is not a complete knowledge about the molecular mechanisms that control intracellular pH in spermatozoa.
In this work we developed a novel strategy using image-based flow cytometry to evaluate intracellular pH changes in a large population of cells, at subcelular resolution. Using human sperm loaded withapH-sensitive probe, we compared the intracellular pH (pHi) levels of cells incubated in non-capacitating media(NC)with cells incubated in capacitation conditions (Cap) from 0 to 4 h. We found that only a small sperm population (~15%) becomes more alkalinethan NC.This alkalization occurs immediately upon the addition of capacitationmedia, and remains constant until the fourth hour of capacitation. ThispHiraise occurs in the head and principal piece, but not in the midpiece where the pHiremained constant during all the incubation time used in this work. Notably,sperm incubated in the absence of HCO_3^-did not display the pHiincrease observed in complete capacitationmedia. Mostly likely HCO_3^-contributes to the pHi increase by entering the cell, in order to establishthe protein(s)involved in this HCO_3^-influx, we employeda pharmacological strategy to identify possible HCO_3^-transporters as follows.S0859(antagonist of Sodium-HCO_3^-Cotransporter (NBC)) producesa significant reduction (p<0.05) of the cell population with more alkaline cytoplasmobserved at the beginning of capacitation. Inh172 (antagonist of the Cystic Fibrosis Transmembrane Regulator (CFTR channel)) reduces the capacitation-associated alkalization (p<0.05) observed after >1 h of incubationbut not the initial rise.Notably, the blocking effect of NBC and CFTR was observed mainly in the head and less in the principal piece.Neither S0859 nor Inh172 produced a complete reduction in the pHiincrease, suggesting that more proteins are involved in HCO_3^-influx.DIDS (a general inhibitor of Cl-dependent anion transport), abolishedcompletely the alkalizationof observed in the head (p<0.01)at the beginning of capacitation,the inhibition the alkalization in theprincipal piece(p<0.05) was observed only after 4 hours of incubation. Cl-GBI (antagonist of the proton channel Hv1), completely abolished(p<0.01)the pHiincrease exclusivelyin the flagellumbut not in headat the beginning of capacitation. Finally, we employed a CASA system to perform motility measurements, and found that NBC, CFTR and Hv1 are involved in the development ofhyperactivated motilitybut notintotal motility.
These results indicate an excellent example of a compartmentalizedcell and regionalized regulation processes. Thefunctional relationship between pHiregulation and motility, and its importance in fertilization should be further studied.

Key words: *Capacitation, Image-based flow cytometry, intracellular pH, NBC, CFTR, Hv1.*
This work was supported by DGAPA-PAPIIT IN203116,CONACyT-Fronteras de la Ciencia No. 71, and CONACyT postgrad studies scholarship.

Effect of Ergosterol in the Tyrosine residue of the Bacilomycine D as a basis for studies of their interaction with lipid membranes.

José Francisco Espinosa-Romero, Sathishkumar Munusamy and Carlos Muñoz-Garay
Instituto de Ciencias Físicas, Universidad Nacional Autónoma de México (ICF-UNAM). Avenida Universidad 2001, Chamilpa, 62210 Cuernavaca, Morelos México.

Carlos Muñoz-Garay. Instituto de Ciencias Físicas, Universidad Autónoma Nacional de México (ICF-UNAM). Avenida Universidad 2001, Chamilpa, 62210 Cuernavaca, Morelos México. Phone (777) 329 1802. E-mail address: cgaray.icf.unam@gmail.com

Overview: In order to study the interaction of different model membranes, which contains ergosterol, with Bacilomycine D, we decided to use the tyrosine present in its amino acid sequence as a fluorescence marker. Thus, it was important to characterize the behaviour of the tyrosine in presence of the lipid in the membrane model. We found that ergosterol quenches the fluorescence of free tyrosine in a more efficient way than other compounds reported in literature, whereas cholesterol has a minimum quenching effect. The emission spectra of tyrosine quenched by ergosterol and acrylamide was studied at different temperatures to determine the nature of the quenching. We also calculated the thermodynamics parameters for ergosterol-lipopeptide and acrylamide-tyrosine interactions, such as the difference in free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) using the Van't Hoff equation. We analysed the excitation spectra of the tyrosine, and found that the quenching of ergosterol and acrylamide is an endothermic and spontaneous process. We studied the interaction mechanism of the interaction of ergosterol and cholesterol with the tyrosine and the lipopeptide via NMR.

Relevance: This study sets the basis for understanding the interaction of drugs with tyrosine residues and the lipids presents in biological model membranes.

The cytochrome *b* carboxyl-terminal end is a central regulator of the bc_1 complex biogenesis in *Saccharomyces cerevisiae*

Daniel Flores-Mireles, Aldo E. García-Guerrero, Yolanda Camacho-Villasana, María Guadalupe Lozano-Rosas, Xochitl Pérez-Martínez.
Departamento de Genética Molecular, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Circuito exterior s/n, Ciudad Universitaria, Coyoacán, 04510 Ciudad de México. Tel. (0155) 5622 5662. Correo electrónico: dmireles @email.ifc.unam.mx

The bc_1 complex is essential for the mitochondrial respiratory chain. Complex bc_1 is a dimer and each monomer consists of 10 different subunits were cytochrome *b* (Cob) is the only subunit encoded in the mitochondrial genome (1). The Cob synthesis and assembly in the inner mitochondrial membrane, is a coupled process. For the synthesis of Cob are required two translation activators (Cbs1 and Cbs2) (1) and two chaperons (Cbp3-Cbp6) (2). In addition, Cbp3 and Cbp6 are involved in maduration of Cob, and coordinate synthesis and assembly (2-3). Cob synthesis is reduced if complex assembly is impaired (2-3). This effect is specially strong in mutants lacking subunit Qcr7, one of the two subunits forming the first assembly intermediate with Cob (1).

Cob is a highly hydrophobic protein, however the carboxyl-terminal end (C-ter) is the only soluble portion of the protein facing the mitochondrial matrix. The crystallographic structure of the yeast bc_1 complex shows that the C-ter end closely interacts with the Qcr7 subunit (4). Our hypothesis is that the C-ter end of Cob is important for its maturation and translation regulation, also for correct bc_1 complex and supercomplex assembly.

We created a mutant lacking the last 13 and 8 amino acids from the Cob C-ter end by mitochondrial transformation. Interestingly we found that the C-ter end of Cob is involved in the regulation of its own synthesis, and is important in the correct bc_1 complex assembly, as well as in formation/accumulation of supercomplexes.

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Biochemical basis of PirA*vp*/PirB*vp* toxins-receptor interactions: a galactosamine mediated binding in the epithelial cells of hepatopancreas of shrimp

Victorio-De Los Santos Marcelo1; Vibanco-Pérez Norberto2; Zenteno Edgar3; Cano-Sánchez Patricia4; Soto-Rodríguez Sonia1 1) Laboratorio de Bacteriología. Centro de Investigación en Alimentación y Desarrollo, A.C. Unidad de Acuacultura y Manejo Ambiental. Av. Sábalo-Cerritos S/N A.P. 711. Mazatlán, Sinaloa, México. C.P. 82100. marcelo.victorio@estudiantes.ciad.mx 2) Laboratorio de Biología Molecular e Inmunología. Unidad Académica de Ciencias Químico Biológicas y Farmacéuticas. Universidad Autónoma de Nayarit. Tepic, Nayarit, México. 3) Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico City, México.4) Laboratorio de Biología Molecular. Instituto de Química, Universidad Nacional Autónoma de México, Mexico City, México.

ABSTRACT

In the majority of the toxins described it is quite clear that the carbohydrate component itself is intimately involved in toxin binding (*Shigella* toxin, cholera toxin, *E. coli* LT, tetanus toxin, and botulinum toxin), where as in a few cases the role of carbohydrate is less well defined (diphtheria toxin, *Pseudomonas* toxin, pertussis toxin, and E. coli ST). Information on the nature of the receptorbinding domains of the *Vibrio parahaemolyticus* PirA*vp* and PirB*vp* toxins is at this point minimal or nonexistent. We investigated the macromolecular interactions between these two toxins with the membrane component of the Hepatopancreas cells of *Peneaus vannamei.* We found that these two toxins can organize themselves in different oligomerization states and required a galactosamine residue to

INTRODUCTION

Outbreaks of serious diseases inflict serious losses in shrimp farming. From 2015 to present in México and South American, white and grass shrimp production has been a significant loss due to the **acute hepatopancreatic necrosis disease** (AHPND), an emerging challenge to this industry. The pathogenic agent of AHPND is a specific strain of *Vibrio parahaemolyticus* which contains PirA*vp* and PirB*vp* toxins encoded in the pVA1 plasmid.

This toxin has been shown to cause the typical histological symptoms of AHPND in infected shrimps, and in this work, we will focus on our structural understanding of these toxins and their binding to galactosamine residue in the membrane receptor.binding and cause the disease in shrimp.

METHODOLOGY

PirA*vp* and PirB*vp* were purified from broth cultures of 17 hours of isolates of Vp AHPND+

by affinity chromatography in a stroma from Wistar rat erythrocytes. The proteins recombinants were cloned into the plasmid pET28a and pET32c respectively, transformed with *E. coli* BL21 RIIL, induced with 0.5 mM of IPTG and purified with a column Ni-NTA Agarose. The hemagglutinating and inhibition assays were performed in the presence of 2 % erythrocytes suspension in PBS pH 7.4 and different mono and polysaccharides.

RESULTS AND DISCUSSION

In the present report, we identified that PirB*vp* mainly recognized a molecule or receptor in the membrane of red cells of rat. The specificity of this toxin recognition was confirmed by inhibition of protein interaction with galactosamine suggesting that this interaction was mediated through recognition of membrane specific carbohydrates. Also, this result strongly suggested that PirB*vp* is a lectin encoded by pVA1 plasmid of *Vibrio parahaemolyticus*. Currently we are working in recognized a receptor in the membrane of the hepatopancreatic epithelial cell of shrimp and the interaction between PirA*vp* and this novel lectin. Some lectins interact with glycosylation sites in their own membrane cells and regulate intracellular pathways, leading to oxidative burst, among other immune mechanisms. The results of the present study strongly suggest that PirB*vp* and PirA*vp* participates in the egulation of intracellular signaling mechanisms causing the massive sloughing of tubule epitelial cells through a specific receptor.

Growth and reserve lipids production with the non-pathogenic *Acinetobacter baylyi* strain ADP1 using different carbon sources

Karina J. Salcedo Vite1, Juan C. Sigala Alanís2, Daniel G. Segura González1 and Alfredo Martínez Jiménez1. 1Universidad Nacional Autónoma de México – Instituto de Biotecnología. Cuernavaca, Morelos, 62210, México. 2Universidad Autónoma Metropolitana, Unidad Cuajimalpa, Ciudad de México 05348, México. kvite@ibt.unam.mx

Acinetobacter baylyi ADP1, a non-pathogenic Gramm-negative bacterium, possess characteristics for basic and applied research in the fields of bioremediation and industrial production, such as natural transformation, fast growth in mineral media with acetate[1] and reserve lipids production: wax esters and triacylglycerols. Qualitative evaluations to grow on succinate, pyruvate, ethanol[2], 4-hydroxybenzoate and levulinate[3], among other compounds, have been reported. However, no studies have been performed to quantitatively study the growth capacity and accumulation of reserve lipids in relevant carbon sources. This work is aimed to quantitatively determine the capacity of ADP1 to growth in several carbon sources in liquid-mineral media as well as to characterize the production of reserve lipids under nitrogen limitation conditions.

The strain ADP1 did not grow on glycerol, xylose neither lactate. Unexpectedly, ADP1 grew at high concentrations of glucose, pyruvate, acetate and ethanol, with minimal inhibitory concentrations of 350, 70, 15 and 50 g L^{-1}, respectively. The shorter generation time (t_D) values were found with acetate in the range of 0.5 to 1 g L^{-1} (t_D=0.75 h) and ethanol at 5 g L^{-1} (t_D =0.74 h). Interestingly, for glucose and pyruvate a constant duplication time was found in a wide range of concentrations: from 0.5 to 50 g L^{-1} (t_D=2.77 h) and from 3 to 10 g L^{-1} (t_D=1.14 h), respectively. Higher wax ester accumulation was found when acetate and pyruvate were used as carbon sources, suggesting that metabolism of organic acids promotes the production of waxes. However, total reserve lipids yield, as fatty acids (COOHs) and fatty alcohols (OHs) per carbon µmol of the substrates, was similar for glucose (COOHs 105.80 and OHs 37.28 µmol mol_{C}^{-1}) and pyruvate (COOHs 94.44 and OHs 63.70 µmol mol_{C}^{-1}), lower for acetate (OHs 45.90 µmol mol_{C}^{-1}) and no production was detected with ethanol (even though ethanol catabolism requires its oxidation to acetate). These results show that ADP1 has a high metabolic capacity to obtain fast growth with several carbon sources and that the metabolism of wax esters to triacylglycerols ratio depends on the catabolized carbon source.

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In vitro evaluation of the phosphate solubilization efficiency of fungal strains isolated from the rhizosphere of bean, corn and tomato plants.

Dorcas Zúñiga Silgado[1]; Jorge Luis Folch Mallol[2]
[1]. *Ph. D Student in Natural Sciences. Universidad Autónoma del Estado de Morelos. Av. University No. 1001, Col Chamilpa, Cuernavaca, Morelos, México. C.P. 62209. Associate Professor. Faculty of Architecture and Engineering. Institución Universitaria Colegio Mayor de Antioquia. Career. 78 N ° 65-46 Robledo, Medellín, Colombia.*
[2]**.** *Thesis Director. Doctorate in Natural Sciences. Head of the Molecular Biology of Fungi Laboratory. Center for Biotechnological Research.Universidad Autónoma del Estado de Morelos. Av. University No. 1001, Col Chamilpa, Cuernavaca, Morelos, México. C.P. 62209.*
Correspondence Author: *jordi@uaem.mx*

Introduction: Some fungi exhibit phytostimulation and phosphate solubilization capacities being an alternative to satisfy the phosphorus requirement in plants from chemically unavailable forms. Our hypothesis is that the phosphate solubilization efficiency is a function of the composition and concentration of the fungal inoculum.

Aim: To evaluate the *in vitro* efficiency of phosphate solubilization of native fungal strains isolated from rhizosphere and rhizoplane of beans, corn and tomatoes.

Methodology: From the rhizosphere and rhizoplane of beans, corn and tomato plants, 31 fungal morphotypes were isolated. The phosphate solubilization efficiency was evaluated in three solid media and three liquid media with and without phosphate rock (PR). Relative solubilization efficiency (RSE), bioacidification and phosphate solubilization were determined. At the *in vitro* level, the compatibility of the strains was evaluated by means of the Test of Confrontation. Growth rate, compatibility or incompatibility controls were performed through the observation of the presence or absence of a defense barrier. Three of nine phosphorus solubilizing strains (*Mortierela* (M), *Aspergillus* (A), and *Trichoderma* (T)) were selected for simple, dual and triple evaluation in three different concentrations (10_4, 10_6 y 10_8 spores mL^{-1}). By means of phosphate solubilization kinetics the concentration of soluble P and the final pH of the medium were quantified.

Results and Discussion: The interaction between (i) composition and (ii) inoculum concentration on the *in vitro* dissolution efficiency of PR was highly significant ($p \leq 0.01$). The effect of bioacidulation on the *in vitro* solubilization of PR showed significant differences ($p \leq 0.05$). Simple inoculation with (M) at the rate of 10_8 spores mL^{-1} was significantly better ($P \leq 0.05$) with respect to other treatments. Dual inoculation (MA) at the rate of 10_4 spore mL^{-1} was more efficient ($P \leq 0.01$) for the variables described between treatments. Triple inoculation (MAT) decreased P concentration ($H_2PO_4^-$) in relation to the other treatments.

Conclusions: The concentration of P in the culture medium depended on the composition and density of the inoculum. The pH of the media was inversely proportional to the amount of soluble P. Therefore, it is likely that the main mechanism of PR dissolution is the production and excretion of organic acids by the strains. In addition, dual inoculation showed a synergistic effect between treatments. Not so the triple inoculation, given a possible antagonistic effect due to competition for phosphorus as a limiting resource in the system.

Folates and N2 fixation: Compartmentalized distribution of folates derivatives in mitochondria, plastids, and *Rhizobium* from *Phaseolus vulgaris* nodules in relation with N2 fixation levels

Wendy Judith Berdeja Zamudio, Jacob I. Bañuelos Trejo, Perla A. Ramos Parra, Jorge Benavides Lozano, and Rocío I. Díaz de la Garza.
Tecnólogico de Monterrey, Escuela de Ingeniería y Ciencias, Ave. Eugenio Garza Sada 2501, Monterrey, N.L., México, 64849

Nitrogen fixation process in legumes is carried out through symbiosis with nitrogen-fixing organisms known as Rhizobacteria. During symbiosis the bacteria triggers nodules formation in the roots suffering biochemical and morphological changes to become a bacteroid, an organism capable of fixing atmospheric nitrogen (N_2), in exchange the plant provides the necessary nutrients for the symbiont. In tropical legumes such as common bean (*Phaseolus vulgaris*) the N_2 is converted into ammonia (NH_3) by the bacteroid and metabolized into ureides by the plant. Ureides production is supported through the *de novo* purine biosynthesis, which is complex and compartmentalized, it occurs mainly in mitochondria and plastids of the infected cell. *De novo* purine biosynthesis requires folate derivatives in some reactions to accomplish nitrogen fixation. Tetrahydrofolate (THF) and its derivatives, known as folates, are enzyme cofactors involved in the one carbon (1C) metabolism. 1C metabolism impacts several anabolic reactions, such as biological methylations, and amino acid (Met, Gly, Ser), purine and thymidylate biosynthesis. In tropical legumes during symbiotic nitrogen fixation, *de novo* purine biosynthesis increases significantly to support ureides flux; therefore it was hypothesized that folate pools may increase considerably to support purine biosynthesis. Folate content was quantified in root nodules of two different varieties of common bean, folates hyperaccumulate in these organs in the order of >60 times higher than roots and higher than any other reported plant tissue. Nothing is known about folate role(s) during nitrogen fixation. To further characterize folates in nodules, as they are produced and used in several plant compartments, and 1C metabolism is highly compartmentalized; folate contents were measured in isolated mitochondria, plastids and bacteroids of nodules with different levels of N_2 fixation. This was achieved by inoculating *Rhizobium* strains with different fixation capacities*:* CE3, NifA-, PMR4, and *R. giardini* in *P. vulgaris* Negro Jamapa plants. Density gradient centrifugation was used to fractionate nodule subcellular compartments and bacteroids. Enzymatic reactions were carried out to verify the integrity of the organelles and cross contamination. *Rhizobium* folate contents were measured in different fractions of isolated bacteroids (transitioning bacteria and bacteroid) and compared to *in vitro* cultured bacteria. We observed that folate levels in bacteroids increased according with the age of the nodule and the distribution of folate species were different. Bacteroids of young nodules accumulate mainly 5,10-CH_2-THF whereas in bacteroids of 40 days 5-CH_3-THF was the most abundant species. In comparison, *in vitro* cultured bacteria presented a different profile where the most abundant species were THF and 5-CH_3-THF. We also observed that transitioning *Rhizobium* accumulated more total folates with different profiles than the bacteroid. Preliminary data show that mitochondria accumulated mostly 10-CHO-DHF, and plastids accumulated 5-CH_3-THF; mitochondria accumulated more folate amounts than plastids. This work shows that folate metabolism is highly dynamic during symbiosis and hint to a transcendental role in the symbiotic fixation process. The study of folate pools in subcellular compartments is giving us very relevant information about the utilization of these cofactors in the 1C metabolism, their transport among compartments and bacteroid, and their relation with nitrogen fixation during symbiosis.

Folate and ethylene metabolic crossroads: Ethylene modulation of folate pools and methionine synthase expression and activity in climacteric fruit

Rocío Isabel Díaz de la Garza, Carolina García Salinas, and Perla A. Ramos Parra
Tecnólogico de Monterrey, Escuela de Ingeniería y Ciencias, Ave. Eugenio Garza Sada 2501, Monterrey, N.L., México, 64849

Climacteric fruits are characterized by a burst in respiration and ethylene biosynthesis at the onset of ripening. This burst triggers a myriad of physiological and metabolic changes in the fruit that affect both nutritional and organoleptic properties. The postharvest management of climacteric fruit often includes the use of ethylene and 1-MCP, to accelerate and delay, respectively, the ripening process. We have studied for the first time the effects of these processes in the dynamics of fruit folates. Folates are part of the vitamin B complex (B9 vitamin) and their consumption is essential for proper human development from conception to old age. Tetrahydrofolate (THF) and its derivatives, known as folates are cofactors involved in one-carbon transfer reactions known as 1C metabolism, which are essential in all organisms for many anabolic reactions: DNA methylation and the biosynthesis of amino acids (methionine, glycine, and serine), nucleic acids, and Sadenosylmethionine (SAM). In plants, folates are needed during photorespiration and chlorophyll synthesis; SAM, is also required for the synthesis of plant-derived metabolites such as ethylene, nicotinamide, and polyamines.

Folate profiles and contents from climacteric fruits were characterized during postharvest ripening using or not exogenous ethylene. The onset of ripening of non-ethylene-induced papaya, tomato, banana and avocado fruits consistently resulted in lower total folates as compared to the mature green stage. On the other hand, ethylene treatment affected significantly folate pools in the fruit differently, causing, respectively, a 24% and 51% increase in ripe tomatoes and bananas, a 26% decrease in papayas, and no change in avocados compared to non-treated ripe controls. Interestingly, ethylene treatment always affected the accumulation of 5-methyl-THF (5-Met-THF) in all fruits. This folate derivative is involved in ethylene biosynthesis: Methionine synthase (MS) transfers the methyl group of 5-Met-THF to homocysteine to form methionine. Methionine forms SAM, which is the substrate of aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS); ACC is oxidized by ACC oxidase (ACO) to form ethylene. Both enzymes are regulated at the transcriptional level by ethylene.

The proposed crossroad between folate and ethylene metabolism with MS as the interjection point of both biochemical pathways was further studied in papaya fruit. Ethylene and 1-MCP were applied to mature green papayas and folates were measured along with MS gene expression and activity. Levels of expression of the two MS isoforms analyzed positively correlated with ethylene levels and also with the expression of ACO1, a wellknown ethylene-responsive gene. Moreover, MS activity presented the same pattern of correlations. In doing these measurements, we were also able to provide evidence of *in vivo* MS substrate preference for the first time in plants: MS seems to prefer 5-Met-THF with a polyglutamyl tail (PG) of 5 to 12 glutamate residues. Folylpolyglutamyl synthetase (FPGS) and gamma-glutamyl hydrolase (GGH) are the enzymes regulating the length of the folate PGs, the expression of both increased in ethylene treated fruit. Folate PG tails also presented differences during fruit ripening and treatments. Gaining knowledge about this folate and ethylene cross regulation can help to design strategies for increasing folate contents in postharvest fruits to improve human nutrition and also to shed light into the role of folates in fruit ripening and other ethylene-related developmental and metabolic processes.

The Intragenic Method for Maize Improvement: Drought Tolerance and Improved Photosynthesis.

Verónica Garrocho Villegas[1], Víctor Arturo González Hernández[2] and Estela Sánchez de Jiménez[1].
[1] Departamento de Bioquímica, Facultad de Química, UNAM, Ciudad de México, C.P.04510.
[2] Programa de Recursos Genéticos y Productividad, Colegio de Postgraduados. Km. 36.5 Carretera México-Texcoco, Montecillo, Texcoco, Estado de México, C. P. 56230. **e-mail: estelas@unam.mx,**

In Mexico conventional crops genetic breeding is done by recurrent selection of phenotypes showing favorable characteristics when growing under field conditions. This methodology has proven to be effective in maize, but it renders significant results after many selection cycles. Alternatively, molecular biology and biotechnology methods, have allowed important advances in genetic crops improvement in a shorter time. Maize, rice and wheat, among other crops, have been improved through the insertion of genetic units designed in molecular vectors. This process allows to generate plants with desirable genetic traits. However, the method presents drawbacks when it is applied in species of agricultural interest, since DNA sequences from virus and other microorganisms are part of the vectors, in these conditions the genome of the improved plant is altered by these exogenous genes. To solve this problem, new methods have been developed in order to prevent the insertion of genes from species that are not sexually compatible into the genome of the plant under study. One example is the intragenic method, which modifies gene expression using only DNA sequences selected from the genome of the same plant species to design the constructions. In addition, the current agronomic knowledge, provides a vast source to detect genes in selected genotypes. Therefore, a combination of the intragenic improvement and the agronomic knowledge provides the means for this type of improvement. Furthermore, considering that climate change predicts a significant decrease in maize and other cereal yields because the increment in global temperature and drought periods. Since drought stress affects maize particularly by inhibiting floral development and causing fertilization failures provoking a decrease in productivity. This is important in Mexico, because nearly 80% of the maize cultivation is done in dryland conditions and depends solely on the rainy season. This situation threat, the country food security, therefore, ensuring the photosynthetic rate and improving the grain formation even in drought conditions is essential. This work presents the strategy to breed maize plants that have higher photosynthetic rate and tolerance to drought stress with an approach to innovate in agriculture, using biotechnological tools, with the aim of conserve and improve Mexican maize genotypes.

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Antagonistic activities analysis of *Trichoderma* spp. isolates over *Fusarium graminearum,* fusariosis causal agent on *Triticum aestivum* (wheat)

Kate Manuela López Hernández, Patricia Abigail Larios Melgarejo, Mauricio Nahuam Chávez Avilés. Instituto Tecnológico Superior de Ciudad Hidalgo. División de Ing. Bioquímica Lab. de Bioquímica y Biología Molecular Av. Ing. Carlos Rojas Gutiérrez #2120 Fracc. Valle de la Herradura, C.P. 61100. nchavez@itsch.edu.mx.

Wheat (*Triticum estivum*) is a perennial herbaceous plant, taxonomically classified into the *Magnoliophyta* division belonging to the family of grasses. Michoacán ranks third nationally in wheat production with 29 thousand Tons per year. However, production losses have been increasing by different factors, one of these are the diseases caused by phytopathogenic fungi. In this sense, *F. graminearum* attacks wheat crops causing fusariosis or white spike disease in the grains, chemical pesticides have been used to control the disease, which are the main cause of water contamination and cause health problems in humans due to its toxicity degree, therefore, biocontrol is currently being explored as an alternative to eradicate the development and proliferation of this microorganism. In the present investigation, the antagonistic capacity of three strains of *Trichoderma* spp. 1, 2 y 3 was analyzed, by means of tests in dual system and of volatile compounds in Agar Papa and Dextrosa (PDA) and Luria-Bertani (LB) medium against six strains of *F. graminearum* (2, 3, 4, 5, 7 y 9) normalizing the growth of both microorganisms. For the evaluations in dual systems in PDA medium, the maximum inhibition percentage was 52, generated by *Trichoderma* T2, followed by *Trichoderma* T1 and *Trichoderma* T3 with 49 and 45 %, respectively. In the evaluations of volatile compounds in PDA medium, the maximum percentage of inhibition was 29 % on the part of *Trichoderma* T1 followed by *Trichoderma* T3 and T2 with 24 and 23 %, respectively, these evaluations were carried out with the strains of *F. graminearum* 2, 4 and 9. In the dual system assays in LB medium, the strains of *F. graminearum* 3, 5 and 7 were used and the maximum percentage of inhibition was 9.5 % for *Trichoderma* T1 followed by *Trichoderma* T2 and *Trichoderma* T3 with 8% for both strains. The maximum percentage of inhibition in the assays of volatile compounds in LB medium was 51 % by the strain *Trichoderma* T1 followed by *Trichoderma* T3 and T2 with 42 and 33 %, respectively. With which it is concluded that the strains of *Trichoderma* spp. used in this study present biotechnological potential to carry out the control of fusariosis in wheat crops.

Carotenoid/Bacteriochlorophyll ratio during the culture of *Rhodopseudomonas palustris* ATCC1007 at different nitrogen concentration.

Carlos Jeovanny López Romero, Luis Gilberto Torres Bustillos, Edgar Salgado Manjarrez, Elvia Inés García Peñaa,
Unidad Profesional Interdisciplinaria de Biotecnología, Instituto Politécnico Nacional, Av. Acueducto de Guadalupe S/N Barrio la Laguna, Gustavo A Madero, Ciudad de México, 07340, México. a inesppu3@yahoo.com.mx.

In this work, *Rhodopseudomonas palustris* ATCC1007 was used to produce carotenoids (CD) and bacteriochlorophyll *a* (Bchl*a*) under low light conditions (30 µE m^{-2} s^{-1}) provided by white light emitting diodes (LEDs). Two batch cultures were made with two nitrogen sources at two different levels. One culture was cultivated with 1 g L^{-1} of ammonium sulfate and 1 g L^{-1} of yeast extract, and the other with 0.2 and 0.05 g L^{-1} of ammonium sulfate and yeast extract respectively. The carbon source in both cases was sodium acetate (2 g L^{-1}). The culture was carried out in a New Brunswick Bio Flo 110 reactor, with an operation volume of 4 L.

It was obtained that the specific growth rate, specific concentration of pigments ($g_{pigment}$ g_{cell}^{-1}) and the ratio CD/Bchl*a* are not affected by the nitrogen concentration on the medium (at the levels studied in the work). The results also showed that the CD and Bchl*a* production is associated to the bacterial growth. However, after 168 hours of culture the carotenoids concentration decrease abruptly while the biomass still increasing exponentially, and then starts to increase again exponentially. This effect is appreciated in both cultures, which indicates that could be due to other factors that were not considered in the work, which can be environmental or genetics. These changes are still being investigated.

Additionally, in both cultures the ratio CD/Bchl*a* increase slightly the first 168 hours and then decrease abruptly in just 24 hours. Then, it stays constant the rest of the culture. In any case, the ratio always had a value less than 1, indicating that under the culture conditions used in the work Bchl*a* is the pigment that is produced the most.

Finally, the maximum carotenoids specific concentration obtained was 3.7 g_{CD} $g_{biomass}^{-1}$, which is twice the value obtained in other works. This may be because the light intensity used in this work is lower than the used in those works.

BISPHENOL-A ON THE GROWTH AND ENZYMATIC ACTIVITY OF *Aspergillus fumigatus*

[1]Binicio Ramírez-Mendoza, Alberto de Jesus Ortiz-Zamora, Josué Israel Zamora-Zamora, José Luis Torres-García, Gabriela Córdoba-Sosa y [1]Miriam Ahuactzin-Pérez.

[1]Autonomous University of Tlaxcala, Faculty of Agrobiology, Degree in Biology, Food Production and Processing Laboratory. Ixtacuixtla, Tlaxcala, Mexico.
E-mail: miriamahuactzin@gmail.com
biny.rm@gmail.com
Phone: 2462248484

Abstract

Bioremediation is the technology that uses the metabolic potential of microorganisms to transform xenobiotics into less complex compounds, of these xenobiotics Bisphenol-A (BPA) is a plasticizer additive present in different environmental matrices by the manufacture of plastic compounds. The objective of the study was to determine the effect of BPA on the growth *of Aspergillus fumigatus* and the activity of laccase enzymes in solid fermentation. Filamentous fungi were isolated from contaminated soils of the Xicohténcatl industrial corridor, Tlaxcala, Mexico, to the fungi that showed the highest radial growth, they were tested for tolerance [0, 20, 40, 60, 80 and 100] mg/L of BPA, from this test *A. fumigatus* showed greater tolerance of up to [80] mg/L, it was grown in three different culture media with [0], [50] and [75] mg/L of BPA to evaluate: specific speed of growth (μ), maximum biomass produced (X_{max}), glucose consumption and enzymatic yields (U/L), ($Y_{E/X}$), ($P=E_{max/h}$), (E_{max}) and $qp=(\mu)*(Y_{E/X})$. The μ, X_{max}, E_{max}, were significantly greater in the treatment of [75] mg/L of BPA. *A. fumigatus* showed acidic pH values in [0] and [50] mg/L and basic from 36 h in [75] mg/L of BPA. The highest laccase activity was 195 U/L at 72 h of growth in the medium of [75] mg/L of BPA. Based on the results, we conclude that *A. fumigatus* used BPA as a secondary source of carbon, and that exposure to BPA induced the production of laccases, enzymes that could be used in bioremediation processes.

Expression of PD1 and TIM3 in the inflammatory peritumoral infiltrate of patients with breast cancer

Miguel Angel Fonseca-Sánchez[1,*] Yanelly Trujillo Cabrera[1], Carlos Lara[2], Manuel Acuña Tovar[2], Joselin Hernández-Ruiz[3], Gloria Queipo[1,4]

1. Departamento de Genética, Hospital General de México Dr. Eduardo Liceaga.
2. Servicio de Oncología, Hospital General de México Dr. Eduardo Liceaga.
3. South Texas Diabetes and Obesity Institute, University of Texas Rio Grande Valley.
4. Facultad de Medicina, Universidad Nacional Autónoma de México.
5.

*Correspondence: Miguel Angel Fonseca-Sánchez: mfonseca_79@hotmail.com Hospital General de México. Dr. Eduardo Liceaga. Dr. Balmis 148 Colonia Doctores CP.01120. México. D.F. México.Tel. 2789 - 2000 Ext. 5802

Background: Immune system plays a major role in neoplastic diseases. The tumoral immune infiltrate has been extensively studied in several neoplasias, however the immune changes of PD1 and TIM3 in breast cancer are less known. The aim of this study was to evaluate the expression of immunoregulator molecules PD1 and TIM3 in TCD8 lymphocytes on infiltrate breast tissue biopsies of patients with breast cancer in different clinical stages, in order to assess the immune response of different tumor loads in accordance with the clinical stage, and to compare it with that of patients with benign tumors.

Methods: We analyzed blood of 55 patients. 49 patients with breast cancer in different stages (Stage I and II: 24 patients, Stage III and Stage IV: 25 patients), 6 patients with benign breast lesions. The expression of PD-1, TIM3, CTLA-4 and CD28 on TCD8 lymphocytes was analized by immunohistochemistry and compared in the different groups. ANOVA and Kruskall-Wallis analysis was performed.

Results: First, we identify up-regulation of TCD8 lymphocytes only in initial stages of breast cancer and similar expression in all stages without differences between them. Second, Up- regulation of PD1 in T CD8 cells on the compartment stromal tumor of cancer patients was identified mainly in stage I patients but it stabilizes in later stages. Finally, The expression of TIM3 wasn´t different between any cancer stage and control groups.

Conclusions: The identification of immunomodulatory molecules such as PD-1 and TIM3 in tumoral immune infiltrate cells of breast cancer patients is feasible and the expression of these molecules differ between healthy volunteers, and different stages of breast cancer patients This becomes relevant to determine the best time in the course of the disease to give targeted immunotherapy, as well as to identify those patients that could get a greater benefit from this treatment and a better prognosis.

Activation of energy metabolism by estrogen in human breast carcinoma

Silvia Cecilia Pacheco Velázquez, Ingrid Itzayanna Ortega Mejía. Departamento de Bioquímica, Instituto Nacional de Cardiología "Ignacio Chávez", Juan Badiano #1 Col. Sección XVI Del. Tlalpan C.P 14080 México D.F. Phone 5573 2911 ext. 25307. E-mail: suerte11@hotmail.com

Introduction: Worldwide, estrogen receptor-positive breast cancer (ER+BC) has the highest prevalence. Despite having a specific therapy (tamoxifen), side effects lead to treatment interruption; thus, recurrence and metastasis are frequent. Moreover, prolonged estrogen exposure is a risk factor for breast cancer development and progression. ERs are transcriptional factors whose target genes are involved in differentiation, inflammation, proliferation, and regulation of energy metabolism.

With the aim of proposing novel therapeutic strategies, we determined the prevalent energetic pathway (glycolysis or oxidative phosphorylation; OxPhos) of the ER+BC cell line, MCF7, in the presence of 17β-estradiol (E2). The latter, considering that previous studies (i.e., in biopsies, xenotransplants, and monolayer cultures) report an increase of glycolytic and mitochondrial enzymes (at mRNA and protein content). However, the metabolic transition after the activation of ERs has not yet established.

Results. MCF7 was grown in increasing concentrations of E2 (0.1, 1, 10, 100 nM) in monolayer cell culture and multicellular tumor spheroids (MCTS, a model that mimics the first stages of an avascular tumor). In both models, E2 at 10 and 100 nM promoted growth (20%), so further experiments were done in these conditions:

For monolayer culture, E2 augmented slightly (0.3-2 times) the protein content of glycolytic (HKII and LDH) and mitochondrial (ND1) enzymes; this led to an increase of glycolytic flux (3.3 times). However, total respiration decreased by 30% without affecting OxPhos, which remained constant.

In the MCTS, E2 augmented (1-4 times) the content of several glycolytic (GLUT1, HKI, and HKII, LDH-A) and mitochondrial (2OGDH, ND1, COXIV, ATPs) enzymes. Consistently, a considerable increase in glycolysis was observed (2.3 times), but it was not so impressive for OxPhos (0.5 times). These data suggest that E2 will favor glycolysis in higher proportion, inducing a Warburg phenotype in ER+BC.

LPS-induced cancer progression on triple negative breast cancer cells via nitric oxide signaling pathway mediated by TLR4/NF-ƙB

Karina Ruiseco-Flores, María Julieta López-Cuevas, Eduardo Monjaraz-Guzmán
Instituto de Fisiología, Benemérita Universidad Autónoma de Puebla
14 sur 6301, CU, San Manuel, Puebla, Pue. CP 72570. (222) 229-55-00 Ext. 7311, 7340
ruiseco_0385@hotmail.com

Toll-like receptors (TLRs) are members of the interleukin-1 receptor (IL-1R) superfamily, play a crucial role in the inflammation and innate host defense against invading microorganisms. Toll-like receptors (TLRs) have garnered an extraordinary amount of interest in cancer research due to their role in tumor progression, invasion, survival, and metastasis. Moreover, nitric oxide (NO) is a multifunctional gaseous molecule that is generated by cancer, stromal and endothelial cells and plays a multifaceted role in cancer biology through multiple mechanisms. Accumulating evidence suggests that NO signaling is involved in multiple aspects of breast cancer, including proliferation, migration, invasion, angiogenesis and response to chemotherapy. However, little research has investigated the role LPS-dependent nitric oxide signal pathway activation in breast cancer progression.

This study investigated the expression and biological role of nitric oxide signal pathway in human breast cancer metastasis. MDA-MB-231 are human breast cancer cell lines with high metastatic potential. Using lipopolysaccharide (LPS) to stimulate MDA-MB-231 cells, TLR4 activation notably up-regulated expression inducible nitric oxide synthase (iNOS) and nitric oxide production. LPS enhanced migration of MDA-MB-231 cells by transwell assay and wound healing assay, through NF-κB/iNOS-dependent mechanism. Moreover, LPS was capable of increasing MDA-MB-231 aggressive breast cancer cell proliferation as indicated by Ki-67 mRNA expression and BrdU (5-bromo-2'-deoxyuridine) incorporation assay.

These findings indicated that NF-κB/iNOS/NO signaling, activated by LPS, may participate in the progression and metastasis of human breast cancer and provide a new therapeutic target.

Antiproliferative effect of molecular iodine and cyclophosphamide in acute lymphoblastic leukemia cells

Soria-Ornelas Maritza L, Delgado-González Evangelina, Aceves Carmen
Instituto de Neurobiología, Universidad Nacional Autónoma de México, Juriquilla, Juriquilla Qro. 76230, México.
e-mail: ibq.mlso@gmail.com, caracev@unam.mx

Acute lymphoblastic leukemia (ALL) is the most common neoplasm in children 2-5 years of age. Although in recent years there has been a considerable improvement in the survival rate, 30% of cases fail associated with poor antineoplastic response and intolerance to treatment. Currently, multiple efforts are being made to identify and characterize therapeutic targets for the development of novel antineoplastic therapies that decrease chemoresistance and generate less toxicity. In this regard, several research groups have shown that the molecular iodine (I_2) supplement exerts antiproliferative effects in several cancer cells, inducing their differentiation and restoring sensitivity to conventional drugs. In preclinical (canine and murine models) and clinical trials (women with breast cancer), it has been shown that in combination with traditional antineoplastic treatments, the I_2 supplement exerts synergetic antineoplastic actions and attenuate side effects. In this work, we analyze the impact of I_2 supplement alone and in combination with cyclophosphamide whose is one of the most frequent drugs used in this neoplastic disease. Jurkat E6-1 cell line, a model of ALL T-cell, was treated with: 1) I_2, 2) 4-hydroperoxicyclophosphamide (4-HC) (an active metabolite of DNA alkylating agent cyclophosphamide (CFF), or 3) both components in combination (I_2/CFF) by 72 hours. The half maximal inhibitory concentration (IC_{50}) of each drug was 275 µM and 0.71 µM for I_2 and CFF, respectively. The combination treatment allowed to reduce CFF dose in 3-fold (200 µM I_2/0.25 µM CFF), suggesting an additive or synergic effect. Molecular analysis showed that I_2 inhibition is accompanied by significant increases in the expression (RT-qPCR) and protein (Western blot) of peroxisome proliferator-activated receptor gamma suggesting the participation of these receptors in the effects mediated by I_2.

Keywords: molecular iodine, acute lymphoblastic leukemia, cyclophosphamide, adjuvant effect.

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Analysis of irisin protein expression, in infiltrating breast cancer, in postmenopausal women with different body mass indexes

María Elena Tejeda Hernández1, Patricia Canto1, Alberto Tenorio2, Ramón Coral-Vázquez3, Verónica Bautista Piña2, Claudia Vega García5, Alejandro Zentella Dehesa4, Marcela Leal García1, Juan Pablo Méndez1
1Unidad de Investigación en Obesidad, Facultad de Medicina, UNAM. 2Instituto de Enfermedades de la Mama FUCAM. 3Sección de Estudios de Posgrado, Instituto Politécnico Nacional & C.M.N. "20 de Noviembre, ISSSTE. 4Instituto de Investigaciones Biomédicas, UNAM. 5Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán", Vasco de Quiroga 15, Belisario Domínguez, 14080 Ciudad de México, CDMX.
Tel. 01 55 5487 0900, ext. 5068.
Correo: elenatejedah@gmail.com

Background: Breast cancer (BC) is a global public health problem, constituting the most common malignancy and the leading cause of cancer-related death among women worldwide. This cancer is considered a multifactorial disease, where genetics and risk factors play a key role in its onset. Obesity constitutes an important risk factor for BC in postmenopausal women. Obesity leads to the altered expression of diverse factors including several adipokines that promote the survival of cancer cells, metastasis, angiogenesis and decreased apoptosis. Recently, it has been demonstrated that irisin, a myokine, a protein secreted by muscle and visceral, as well subcutaneous adipose tissue, decreases the number of malignant mammary epithelial cells, their migration and their viability when compared with non-malignant cells. Besides, this myokine is expressed in BC tissue.

Aim: Investigate the expression of irisin, in infiltrating breast tumor tissue, in postmenopausal women with different body mass indexes. Besides, we analyzed the *in vitro* expression of irisin in different cell lines of BC.

Subjects and Methods: Sixty postmenopausal Mexican-Mestizo women (45-89 years of age) with BC, who underwent mastectomy or breast conserving surgery were studied. Three groups were formed according to body mass index (BMI). Immunohistochemistry (IHC) of irisin, using a specific antibody, was performed in the paraffin-fixed tumor tissue, as well as in BC cell lines (MCF-7, BT-474, SKBR-3 and MDA-MB-231). Percentage of tumor cells was analyzed by ImageJ software. Kruskal-Wallis test was employed for non-parametric values and a $p<0.05$ was considered significant.

Results and discussion: According to WHO criteria among the 60 women, 20 had a normal BMI, 20 were overweight and 20 had obesity. The IHC showed a lower expression of irisin in BC tissue of women with obesity when compared with women with normal BMI ($p <0.009$). We did not observe differences of the expression in overweight patients. In addition, the *in vitro* study revealed that the four BC cell lines analyzed expressed this protein.

Conclusion: To our knowledge, this constitutes the first report where the expression of irisin, in postmenopausal women, with different BMI and BC has been analyzed. We observed a lower expression of this myokine in BC tissue in postmenopausal women with obesity. Besides, we demonstrated that this protein is expressed, by IHC, in four different lines of BC.

Over-elongation of centrioles in cancer promotes centriole amplification and size deregulation, as recurrent features of cancer cells.

Adan Guerrero[1,2], Gaelle Marteil[2], Andre F. Vieira[3,4,] Bernardo P. de Almeida[5,6], Pedro Machado[2], Susana Mendonca[2,3,4], Marta Mesquita[7], Beth Villarreal[8], Irina Fonseca[2], Maria E. Francia[2], Katharina Dores[2], Nuno P. Martins[2], Swadhin C. Jana[2], Erin M. Tranfield[2], Nuno L. Barbosa-Morais[5], Joana Paredes[3,4,] David Pellman[11], Susana A. Godinho[9], Monica Bettencourt-Dias[2].

1Laboratorio Nacional de Microscopia Avanzada, Instituto de Biotecnologia, Universidad Nacional Autonoma de Mexico (UNAM), Cuernavaca, Morelos 62210, Mexico.

2 Instituto Gulbenkian de Ciencia, Oeiras 2780-156, Portugal.

3 I3S - Instituto de Investigacao e Inovacao em Saude, Universidade do Porto, Porto 4200-135, Portugal.

4 IPATIMUP - Instituto de Patologia e Imunologia Molecular, Universidade do Porto, Porto 4200-135,Portugal.

5 Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon 1649-028,Portugal.

6Departamento de Ciencias Biomedicas e Medicina, Universidade do Algarve, Faro 8005-139, Portugal.

7 Instituto Portugues de Oncologia de Lisboa, Lisbon 1099-023, Portugal.

8 Novartis Institutes for BioMedical Research, Boston, MA 02139, USA.

9 Dana-Faber Cancer Institute, Boston, MA 02215-5450, USA.

Correspondence to A.G. (email: adanog@ibt.unam.mx)

Centrosomes are the core of the major microtubule organizing centers of animal cells. Deregulation in their number and size have been shown that occurs in cancer, however, the incidence of these abnormalities, origins and consequences are poorly understood. By means of automated multidimensional confocal microscopy, and the development of image processing tools, we screened the NCI-60 panel of human cancer cell lines to systematically study defects in centriole number and structure. We showed that centriole amplification is widespread in cancer cell and reigns in aggressive carcinomas, such as breast cancer. Our screening revealed that centriole size deregulation is another recurrent feature of cancer cells. Severe centriole over-elongation promotes centriole amplification through both centriole fragmentation and ectopic procentriole formation. This study supports centriole amplification and size deregulation as recurrent features of cancer cells and uncovers novel causes and consequences of those abnormalities.

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Marteil, G. et al., 2018. Over-elongation of centrioles in cancer promotes centriole amplification and chromosome missegregation. *Nature Communications*, 9, 1258.

VASOINHIBINS PROMOTE APOPTOTIC CELL DEATH IN HIPPOCAMPAL NEURONAL PRIMARY CULTURES

Rodrigo Manuel Aroña[1], Edith Arnold[1,2], Fernando Macías1, Miriam Ulloa[1], Josué Rivera[1], Carmen Clapp[1], Gonzalo Martínez de la Escalera[1].

[1]Department of Celular and Molecular Neurobiology; 2CONACYT-Neurobiology Institute, Universidad Nacional Autónoma de México, Campus UNAM Juriquilla, Querétaro, México.

Vasoinhibins (Vi) are a family of peptides derived from prolactin that have been shown to act on endothelial cells blocking angiogenesis via inhibition of proliferation and inducing vaso-obliteration through apoptosis. Similarly to other angiogenic regulatory factors, Vi can modulate some functions of the Nervous System. Vi act in the Central Nervous System promoting anxiety and depression behaviors. Additionally, Vi suppress the neurotrophic effects of vascular endothelial growth factor (VEGF) in primary sensory neurons. Both, anxiety and depression, as well as the inhibition of VEGF actions have been associated with hippocampal neurodegeneration. Thus, in the present study we explored whether Vi affect hippocampal neurons. To explore the actions of Vi on neuronal cells, primary hippocampal neurons were isolated from the brain of E16 mice and cultured on plates or coverslips treated with poli-L-lysine. On DIV1 hippocampal cultures were treated with increasing concentrations of Vi (5, 10, 20, 40 nM) for up to 72 hours (DIV1-DIV4) or with a concentration of 20 nM for up to 16 and 24 hours. Incubation of hippocampal cultures with Vi reduced the cell number in a dose-response manner, evaluated by immunocytochemistry for βIII-tubulin, a neuronal marker. Vi induced the activation of caspase 3 at 16 and 24 hours after treatment, evaluated by immunocytochemistry for cleaved caspase 3. Moreover, Vi increased the number of apoptotic cells and the expression of genes involve in apoptosis, such as CASP3, the transcription factors FOXO1 and FOXO3, and the members of the Bcl-2 family BAD, BAX, BIM and PUMA, evaluated by TUNEL and qRT-PCR, respectively. Altogether these findings show that Vi are capable to induce the apoptosis of hippocampal neurons and suggest that it occurs via the activation of the apoptotic mitochondrial intrinsic pathway, remaining to demonstrate the role of transcription factors FoxO1 and FoxO3 in this mechanism.

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Effect of oxidative stress on the P2X7 purinergic receptor and the glycogen synthase kinase transcriptase, in the hippocampus of the rat exposed to low doses of ozone

Velázquez-Pérez Raúl*, Rodríguez-Martínez Erika*, Valdés-Fuentes Marlen**, Selva Rivas-Arancibia*
*Departamento de Fisiología, Facultad de Medicina. Universidad Nacional Autónoma de México. CP 04510. Tel. 56232500, e-mail srivas@unam.mx
**Departamento de Investigación. Tecnológico de Estudios Superiores de Huixquilucan. Estado de México. México. CP 52773. Tel 82881130

Abstract: Ozone exposure at low dose causes oxidative stress and induces a progressive neurodegeneration in hippocampus of rats when they are chronically exposed to this gas. Adenosine 5'triphosphate (ATP) can act as a mediator in intercellular communication between neurons and glial cells. In a chronic oxidative stress state, there and increase in extracellular ATP levels, which promotes a modification in the regulation of cellular energy metabolism, since it can affect the proteins that are involved in these processes, as well as the messenger RNAs that transcribe for these proteins. The objective of this work was to study the effect of chronic low ozone dose on the P2X7 receptor expression as well as the expression of GSK3β in rat hippocampus. For this purpose, 36 Wistar male rats were used, with free access to food and water, housed individually, and randomly divided into 6 groups. Each group received one of the following treatments: group 1: control exposed to ozone-free air, group 2, 3, 4, 5 and 6 exposed to ozone for 7, 15, 30, 60 and 90 days respectively. The ozone exposure was for 4 hours daily at 0.025 ppm. Two hours after the end of the treatment, the rats were deeply anaesthetized and processed for the PCR, and western blot techniques. Gene expression was evaluated by isolating total RNA from hippocampus, the amount and quality of RNA were estimated spectrophotometrically. Amplification was performed in triplicate in the MIC real-time PCR detection system (MIC®). Using specific primers corresponding to P2X7 and GSK3β sequences. To calculate mRNA gene levels, PCR data were analyzed by the 2-ΔΔCT method, and cycle thresholds were normalized to the housekeeping gene Rps18. The amount of proteins expressed by the hippocampal cells were evaluated semi-quantitatively by western blot. The results showed that the messenger of the p2x7 receptor increases significantly after 15 days of ozone exposure ($p < 0.05$) reaching its maximum at 60 days. The expression of P2X7 proteins also shows a significant increase at 60 days of ozone exposure. Regarding GSK3β, a significant increase was observed ($p < 0.05$) at 30 and 60 ozone exposure, which is also significant in the western blot.

In conclusion, the results indicate that exposure to low doses of ozone causes an increase in the P2X7 ATP receptors, and an increase in the GSK3β protein, which may indicate that the activation of these receptors is altering the signaling pathways in which that enzyme participates.

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Study of the differences between the proteomic profile of the dorsolateral prefrontal cerebral cortex of individuals who died by suicide and of individuals who died from other causes

Manuel Alejandro Rojo – Romero [1], Nora Andrea Gutiérrez – Nájera2, Jonatan Torres – Campuzano [1], Ana Luisa Romero – Pimentel [3], Mirna Edith Morales – Marín[2], Fernando García – Dolores 4, Roberto Cuauhtémoc Mendoza – Morales 4, Alejandra Mendoza – Larios 4, Ernesto Díaz – Otáñez 4, Eli Elier González – Sáenz 5, Humberto Nicolini – Sánchez [2]
1.- Universidad Nacional Autónoma de México, Facultad de Ciencias. 2.- Instituto Nacional de Medicina Genómica (INMEGEN), Servicios de Atención Psiquiátrica (SAP), Secretaría de Salud, Ciudad de México. 3.- Universidad Nacional Autónoma de México, Facultad de Psicología. 4.- Instituto de Ciencias Forense (INCIFO), Tribunal de Justicia de la Ciudad de México. 5.- Hospital Psiquiátrico Fray Bernardino Álvarez, Ciudad de México.
Postal address: Juana de Arco #95. Ins. 502, Col. Moderna. Del. Benito Juárez. CP: 03510. **Telephone number:** (+52) (045) 55 10 16 15 92. **Email address:** ale_fkcod@hotmail.com. **Place where the work did:** Instituto Nacional de Medicina Genómica (INMEGEN)

Objective: To analyze the protein profile of the dorsolateral-prefrontal cortex of individuals who completed suicide.
Background and Aims: According to the World Health Organization (WHO), suicide is the second cause of death in people between 15 and 29 years old. Suicide is a complex phenomenon that involves many risk factors. Currently, proteomics offers the advantage of studying many proteins in the same experiment through high technologies, as mass spectrometry, bringing a complete view of the proteome of a specific biological sample. Proteins play important roles in cell biology, but also alterations of their expression can be involved in the development of many pathologies.
Materials and Methods: We extracted the protein of 5 brains from individuals with completed suicide. We obtained control samples from 4 brains of people who died by other causes. For the protein analysis we made 2D gels and these were analyzed with the PDQuest software powered by Bio-Rad, in order to compare protein gel images. The proteins of interest were identified by mass spectrometry.
Results: We identified proteins that could be involved in the neurobiological function of suicide. These proteins can be related to biological processes as redox metabolism.
Conclusion: Identifying the alterations in the brain proteins of individuals with completed suicide will increase the knowledge of this health problem and could help in its prevention and diagnosis.

Prolactin protects rat cortical astrocytes against oxidative stress.

Miriam Ulloa[1], Fernando Macías[1], Rodrigo Manuel Aroña[1], Carmen Clapp[1], Gonzalo Martínez de la Escalera[1], Edith Arnold[1,2].
[1]Department of Celular and Molecular Neurobiology; [2]CONACYT, Neurobiology Institute, Universidad Nacional Autónoma de México, Campus UNAM Juriquilla, Blvd. Juriquilla 3001, Querétaro, México. CP. 76230, Tel: 4422381028 Correo: earnoldh@gmail.com

Astrocytes participate in brain homeostasis by maintaining synaptic integrity, providing protection and metabolic support for neurons, regulating inflammatory response, and promoting cell survival under oxidant conditions. Several types of stress, injuries and brain diseases induce mitochondrial dysfunction and oxidative stress that lead to astrocyte death. Prolactin (PRL) is a stress-related hormone that limits gliosis and degeneration of the neural retina (Arnold et al JN 2014). In this work, we investigated whether PRL protects cortical astrocytes against oxidative stress and cell death. Primary cultures of cortical astrocytes were isolated from the brain of neonatal rats, and characterized by an immunocytochemistry for GFAP, a marker for astrocytes. The long isoform of the PRL receptor was detected in cortical astrocytes by qRT-PCR. The astrocytes were incubated with increasing concentrations of PRL during 24 h, and exposed to oxidative stress induced by hydrogen peroxide (H_2O_2) for 3 h. PRL inhibited the H_2O_2-induced cytotoxicity in cortical astrocytes in a dose-dependent manner, as evaluated by the MTT assay. In addition, PRL induced an increase in the expression of its receptor and GFAP under oxidantive conditions, as well as an increase in the expression of antioxidant enzymes such as: Mn and Cu/Zn superoxide dismutases, peroxirredoxins 1 and 6, glutathione peroxidase 1 and glutathione S-transferases μ1 under basal conditions in astrocytes, and these changes were exacerbated after H_2O_2-induced oxidative stress. Catalase, glutathione S-transferases μ3 and hemoxygenase 1 expresion increased only by effect of H_2O_2. These changes were evaluated by qRT-PCR. Moreover, PRL increased the total antioxidant capacity of the cultures in basal and oxidative conditions. Finally, PRL receptor activation was determine through the evaluation of phosphorylated forms of STAT5 and STAT3, by Western blot. These results indicate that PRL can act through its receptor, directly on astrocytes to protect them against injuries due to oxidative stress.

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The role of autophagy and cellular senescence during the development of spinal cord and differentiation of motoneurons

Jorge Antolio Domínguez-Bautista and Susana Castro-Obregón
Department of Neurodevelopment and Physiology, Institute of Cellular Physiology, Universidad Nacional Autónoma de México, Mexico City, Mexico.
Circuito Exterior s/n Ciudad Universitaria, Coyoacán, 04510 Ciudad de México. Tel (52) (55) 5622 5676; scastro@ifc.unam.mx

A massive wave of apoptosis that occurs during embryonic development of the spinal cord accounts for clearing 50% of the motoneurons generated in earlier stages. Interestingly, embryos with defective autophagic machinery may develop normally, although the processes involving programmed cell death appear delayed. Thus, it is assumed that additional cellular events may contribute to the elimination of motoneurons. Here we investigate whether autophagy and cellular senescence occur during normal embryonic development, and analyze their potential role in the development of spinal cord and differentiation of motoneurons. Indeed, markers of autophagy and cellular senescence were observed in the developing spinal cord, including the zone where motoneurons are located. A population of cells with phagocytic activity contains markers of autophagy, increased lysosomal function and molecular markers of cellular senescence. Furthermore, the occurrence of cellular senescence is not limited to phagocytic cells, but could be detected in other cell types. We will discuss whether pharmacologic inhibition of autophagy influences the number of surviving motoneurons and whether cellular senescence occurs during normal motoneuron development.

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"Cellular systems proposed as a model for the study of dementia"

Fernanda Anaid Villarreal Reyes; Erick Cuevas Fernández ; Heriberto Manuel Rivera.
Laboratorio de Tecnología de Proteínas de Membrana, Facultad de Medicina, Universidad Autónoma del Estado de Morelos (UAEM). Calle Iztaccíhuatl esq. Leñeros s/n, col. Los Volcanes, Cuernavaca, Mor. CP 62350. m2mrivera@uaem.mx; @ltpm_mx.

Abstract

Dementia is a condition caused by multiple factors of a chronic and progressive nature, characterized by the loss of cognitive and emotional skills. There are other neuropsychiatric manifestations such as motor behavior disorders, depression, anxiety and hallucinations as a consequence of a process of neuronal degeneration mainly associated with this syndrome.

In the 70s, it was shown that senile dementia was indiscernible clinically and neuropathologically at ages younger than 60 years. Therefore, the need to develop new alternatives for differential diagnosis was clear. Currently, dementia is classified as a major cognitive disorder. There are multiple types of dementia that share symptoms and that have their own pathology. The lack of limits between the different types of dementia demonstrates the difficulty of presenting an accurate diagnosis. The World Health Organization classifies dementia into four main types of Dementia: Alzheimer's, vascular dementia, Lewy body disease and frontotemporal dementia, as well as Creutzfeld-Jakob and AIDS dementia. The pathogenic mechanisms of each type of dementia are different, and the cellular models for their study lack the complexity to understand the multifactoriality of dementia.

This work focuses on the study, identification and classification of cellular systems related to dementia as a study model, in order to identify at an early stage the possible development routes and the anatomical compartment in which it is present, in this way to be able to address the different manifestations of dementia and its mechanism of pathogenesis.

The TRPV4 cationic channel regulates the transcriptional activity of β-catenin through a direct interaction modulated by its channel activity.

Refugio García-Villegas1, Heidi Gabriela Espadas-Álvarez1, Angélica Rueda y Sánchez de la Vega2, Jacqueline Martínez-Rendón1, Isabel Larré1, Arturo Matamoros1, Tatiana Romero-García2. Departamento de Fisiología, Biofísica y Neurociencias1 y Departamento de Bioquímica2, CINVESTAV-Zacatenco. Av. Instituto Politécnico Nacional 2508, Col. San Pedro Zacatenco, Ciudad de México CP 07360. Tel.:57473800 ext. 5101. rgarciav@fisio.cinvestav.mx

TRPV4 (*Transient Receptor Potential Vanilloid* 4) protein forms a non-selective cationic channel able to permeate Ca^{2+}, Na^{+} and Mg^{2+}. It is sensitive to hypo-osmotic stimuli, responds to temperature in the range of 25-37°C and to the specific synthetic agonist 4α-phorbol 12, 13-didecanoate (4α-PDD). It has been reported that TRPV4 binds to β-catenin in the adherent junction complex of epidermal keratinocytes. Multifunctional β-catenin protein is an essential component of intercellular adherent junctions as well as a transcriptional coactivator in several signaling pathways including that initiated by Wnt which is critical in embryonic development and tissue homeostasis. Thus, β-catenin transduces signals from the cell surface to the nucleus. In the nucleus, β-catenin binds and activates LEF-1/TCF (lymphoid enhancer-binding factor-1/T-cell factor) and other transcriptional factors leading to transcriptional activation of Wnt targeted genes including c-myc and cyclin D1.
In the present study we report for the first time the expression of full-length TRPV4 channel in the nucleus of intact cells and in isolated nuclei of sparsely seeded Madin-Darby canine kidney (MDCK) renal epithelial cell by using immunofluorescence and confocal microscopy. These results were confirmed by Western blot of nuclear protein fractions and nuclear detection of a transfected Flag-tagged TRPV4 channel. We also demonstrated that activation of TRPV4 with 4α-PDD increases cytoplasmic as well as intranuclear Ca^{+2} concentrations in MDCK cells. Activation of TRPV4 also induces the translocation of the channel out of the nucleus together with β-catenin. Additionally, luciferase reporter gene assays showed that the overexpression of TRPV4 has an inhibitory effect on the transcriptional activity of β-catenin. TRPV4-M680K mutant channel that was unable to permeate Ca^{+2} also inhibited β-catenin transcriptional activity showing that inhibitory effect of the channel do not depend on the influx of Ca^{+2}.
We evaluated with a proximity ligation assays (PLA) the interaction of TRPV4 and β-catenin and we observed a direct interaction of these molecules in both the cytoplasm and around the nucleus in sparsely seeded cells but not in the adherent junctions of confluent cultures. Additionally, the activation of TRPV4 channel disrupts its interaction with β-catenin.
Taken together, our results indicate that the TRPV4 channel is located in the nucleus and function as a transcriptional regulator of β-catenin through a direct interaction modulated by the channel activity.

Using yeast genetic synthetic lethality to study *NPA3* function.

Martín A. Mora-Garcíaa, Tania A. Félix-Péreza, Diana I. Ascenciob, Alejandro Juárez-Reyesb, Lina R. Riego-Ruízc, Alexander DeLunab, Mónica R. Caleraa, and Roberto Sánchez-Oleaa.
aInstituto de Física, Universidad Autónoma de San Luis Potosí, Manuel Nava 6, Zona Univesitaria, C.P. 78290, San Luis Potosí, SLP, Mexico. Tel: (444) 826-2300, ext. 5717. Email: rsanchez@ifisica.uaslp.mx. bLANGEBIO: Laboratorio Nacional de Genomica para la Biodiversidad. cIPICYT, División de Biología Molecular.

In both human and *Saccharomyces cerevisiae* yeast cells the GTPase Gpn1 is required for the nuclear accumulation of RNA polymerase II (RNAPII). Our group previously showed that a strain of *S. cerevisiae* that expresses only a mutant form of Npa3, the yeast orthologue of Gpn1, lacking the C-terminal tail (Npa3ΔC) proliferated normally and, importantly, contained RNAPII exclusively in the cell nucleus. However, this strain showed several phenotypes, which indicates that Npa3 fulfills additional cellular functions to those involved in the nuclear localization of RNAPII. Among these phenotypes was a synthetic lethal interaction with *BIK1*, a non-essential gene that encodes a microtubule-binding protein with mitotic functions. In this work we identified other genes whose inactivation in combination with the *npa3ΔC* mutant gene cause synthetic lethality in a synthetic genetic array analysis. For this purpose we generated a collection of double mutants lacking a specific gene and expressing Npa3ΔC by mating a collection of 4,700 strains of *S. cerevisiae* deficient in a non-essential gene with the *npa3ΔC* strain. 57 genes showed a negative genetic interaction with *npa3ΔC*. These genes participate in a variety of biological processes including autophagy, respiration, translation and stability of the mitochondrial genome, cell cycle, chromatin organization, DNA repair, transcription, processing and transport of RNA, transport and localization of proteins, biosynthesis of glutathione, carbohydrates, amino acids and lipids, and some metal ions such as calcium and manganese homeostasis. These results point to novel avenues of future research to identify the cellular and molecular functions of Npa3, some of which we anticipate will be conserved in human Gpn1.

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The pyrophosphohydrolase RppH is involved in the control of RsmA/CsrA expression in *Azotobacter vinelandii* and *Escherichia coli*

Leidy Patricia Bedoya-Pérez, Luis Felipe Muriel-Millán, Soledad Moreno, Elva Quiroz-Rocha, Nancy Rivera-Gómez, Guadalupe Espín.

Departamento de Microbiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México. Av. Universidad 2001. Col. Chamilpa. Cuernavaca, Morelos. 62210, México Tel: 7777-3291629. espin@ibt.unam.mx, lbedoya@ibt.unam.mx

In bacteria, the 5´-end-dependent RNA degradation is triggered by the RNA pyrophosphohydrolase RppH converting tri/diphosphate to monophosphate transcripts. This study shows that in the soil bacterium *Azotobacter vinelandii,* inactivation of *rppH* gene negatively affected the production of bioplastic poly-β-hydroxybutyrate (PHB) by reducing the expression at the translational level of PhbR, the specific transcriptional activator of the *phbBAC* biosynthetic operon. The effect of RppH on the translation of *phbR* seemed to be exerted through the translational repressor RsmA, as the inactivation of *rsmA* in the *rppH* mutant restored the *phbR* expression. Interestingly, in *Escherichia coli* inactivation of *rppH* also affected the expression of CsrA, the RsmA homolog. The level of the *csrA* transcript was higher and more stable in the *E. coli rppH* mutant than in the wild type strain. Additionally, and in contrast to the *csrA* mutants that are known to have a defective swimming phenotype, the *E. coli rppH* mutant showed a hyper-swimming phenotype that was suppressed by a *csrA* mutation, and the *Av*RppH restored to wild type level the swimming phenotype to the *E. coli rppH* mutant. We propose that in both *A. vinelandii* and *E. coli*, RppH activity plays a role in the expression of the translational repressor protein RsmA/CsrA.
Keywords: *csrA, rsmA*, RppH, 5´-end-dependent RNA degradation, PHB, motility

A long intergenic non-coding RNA is a novel positive regulator of the ABA FIVE-BINDING PROTEIN (*AFP1*) in *Arabidopsis thaliana*.

Dhamar Gabriela Rodriguez Tenorio*, Rosa Angélica Montelongo, Jaime Aportela Corte**, Catalina Arenas Huertero**
*Posgrado en Bioprocesos, Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí.
**Facultad de Ciencias, Universidad Autónoma de San Luis Potosí. Diagonal Salvador Nava Mtz s/n, Zona Universitaria, San Luis Potosí., SLP. México. CP 78290. catalina.arenas@uaslp.mx

Plants frequently have to weather both biotic and abiotic stress. Water stress, for instance, affects many aspects of the physiology of plants, thus, they have evolved protective mechanisms to ensure their survival when threatened by adverse environmental conditions. The phytohormone, abscisic acid (ABA), plays an essential part in acting towards varied range of stresses such like water stress. The transcription factor ABA-Insensitive5 (ABI5) is one of the key regulator of ABA signaling and stress response in Arabidopsis. Potential ABI5-interacting protein, such as the ABI Five Binding Protein 1 (AFP1) attenuates ABA signals by targeting ABI5 for ubiquitin-mediated degradation in nuclear bodies. Recently, an intergenic lncRNA has been identified within the AFP and the Nuclear Transport Factor 2 (NTF2) locus, hence we named as *lincAFP1,* which it is expressed under abiotic stress conditions; however, its function is unknown. In this study, we show the identification of two splicing variants (*lincAFP1.1, lincAFP1.2),* and have functions during abiotic stress in *Arabidopsis thaliana*. Overexpression and deficiency of *lincAFP1,* increases and decreases, respectively, the expression of its adjacent genes (AFP1, NTF2). In addition, plants with a deficiency of this gene showed a hypersensitive phenotype with a delay in the germination under the ABA treatments. Meanwhile, the overexpression of the gene result in plants with an insensitive phenotype to ABA treatment. Our results suggest that, *lincAFP1* acts as a positive regulator of the *AFP1* expression and therefore stimulates the tolerance to ABA stress. We are, now, interested in determining the level on which this regulation is carried out.

Methylation marks on the S-RNase promoter suggest a epigenetic regulation associated with a Gypsy retrotransposon-like sequence

Renata Salcedo Sánchez, Yuridia Cruz-González Zamora, Felipe Cruz García.
Depto. de Bioquímica, Facultad de Química, UNAM.
C.P. 04510, fcg@unam.mx

To avoid inbreeding, angiosperms have evolved with a sophisticated genetic mechanism named self-incompatibility (SI), which is defined as the inability of a fertile hermaphroditic plant to produce zygotes after self-fertilization. SI is genetically controlled by the *S*-locus, which encodes the *S*-Ribonuclease (*S-RNase)* as the female determinant. Pollen rejection response picks on anthesis, a developmental stage when the female determinant and other pistil genes such as *HT-B, 120K* and *NaStEP* reach their maximum transcript accumulation.

Transcriptional regulation is studied from a global perspective that includes regulation in the promoter region and epigenetic marks that coordinate chromatin accessibility. To give light on the components of *S-RNase* transcriptional regulation, we cloned a sequence of approximately 1400 bp and through *in silico* predictions were identified a transcription start site (TSS), with a TATA Box element and several binding sites for transcription factors related to the development of floral organs, response to environmental stress and phytohormones. The DNA methylation pattern of leaf, bud immature pistil and mature pistil were identified. Results gave evidence that leaves had a low level of DNA methylation in the three sequence contexts (CG, CHG, CHH), which suggests that transcriptional regulation could not be directed by DNA methylation. On the other hand, in bud immature pistil DNA methylation was higher in both CG and CHG contexts, a promoter regions feature that is commonly associated with gene silencing, which agrees with the expected results. Contrary, DNA methylation in mature pistil was high and with an notable enrichment in a CHH context, which agrees with that reported in *Zea mays* as "mCHH Islands" that include high levels of CHH methylation upstream to the TSS of highly expressed genes that are close to transposable elements (TE's) and mark the transition from heterochromatin to euchromatin.

Upstream to the *S-RNase* TSS, we also identified an LTR retrotransposon of the *Gypsy* family of approximately 300bp at position -239 upstream the TSS that could be classified as a *TRIM* (terminal repeat retrotransposons in miniature), these sequences do not encode the proteins necessary for retrotransposition and go from 300 bp to 1000 bp. It is known that some of these elements can be found upstream a gen and significantly increase its transcription in a tissue-specific manner.

Currently, we are analyzing chromatin marks on H3 histone and performing DNA methylation analysis on *S-RNase* gene body to identify other regulatory elements that could be relevant in *S-RNase* transcriptional regulation.

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Experimental analysis on the *betIBA* operon and *betT1* gene promoters from *Pseudomonas aeruginosa* PAO1

Erick Jael Palomo Paz[1], Isabel Sánchez Morales[2], Javier Andrés Juárez Díaz[2], and Rosario A. Muñoz Clares[1]*

[1]. Departamento de Bioquímica, Facultad de Química, UNAM. Ciudad Universitaria, Cd. Mx. 04510. [2]. Departamento de Biología Comparada, Facultad de Ciencias, UNAM. Ciudad Universitaria, Cd. Mx. 04510.

*Teléfono: (55) 5622 3718; e-mail: clares@.unam.mx

Pseudomonas aeruginosa is one of the most important bacterial pathogens in plants and animals, including humans. This Gram-negative bacterium has an outstanding ability to grow in an extensive variety of organic compounds and habitats, seeming to be ubiquitous. Furthermore, it is able to grow in hyperosmotic environments, mainly due to its ability to synthesize osmoprotectants, particularly glycine betaine (GB) from choline, which is abundant in many of the infected tissues.

The *betIBA* operon includes the *betI* gene, which encodes the repressor protein of the operon itself, BetI, and the two genes that encode the dehydrogenases involved in the oxidation of choline to GB: *betA*, which encodes choline dehydrogenase, and *betB*, which encodes betaine aldehyde dehydrogenase. Choline is imported by the bacterium either by an ABC transporter or by BetT1, which is encoded by the gene *betT1*. This gene is located adjacent, but divergent, to the *betIBA* operon.The promoter regions for both *betIBA* and *betT1*, which we named *A* and *C*, respectively, are overlapped in the intergenic region, which in addition contain in both DNA strands the BetI repressorbinding site. By *in silico* analysis, we found two more promoter-like domains: promoter *B*, located in the intergenic region upstream the promoter *A* at 164 bp distance, and promoter *D*, located 152 bp upstream promoter *C*. We constructed and analyzed different transcriptional fusions with *lacZ* and found that the four promoters regulate the transcription of both, the *betIBA* operon and the *betT1* gene. Our results suggest that BetI interacts with the DNA forming different complexes in the activated expression state (when bound to choline) or in a repressed state (in the absence of choline). To evaluate this hypothesis, recombinant *Pa*BetI was overexpressed in *Escherichia coli* in order to identify the allosteric site where choline is bound and how this affects its binding to the promoters that we are proposing.

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The role of Voltage-Gated Sodium Channels Subunits in Osteosarcoma Metastasis

Nidia Ednita Beltrán Hernández[1-2], Heriberto Manuel Rivera[1*]

[1]Laboratorio de Tecnología de Proteínas de Membrana, Facultad de Medicina, Universidad Autónoma del Estado de Morelos, Calle Leñeros SN col. Volcanes C.P. 62350 Cuernavaca, Morelos, México
2Programa de Maestría y Doctorado en Ciencias Bioquímicas, Universidad Nacional Autónoma de México-Instituto de Biotecnología UNAM campus Morelos. Av. Universidad 1001 Col. Chamilpa C.P. 62209 Cuernavaca, Morelos, México.
Email: *m2mrivera@uaem.mx

Osteosarcoma is the most common primary bone tumor with an incidence of 8-11 cases/1,000,000 in adolescents. Also, osteosarcoma etiology has been yet unclear for this the most patients are diagnosed in advanced states. Additionally, 90% of patients develop metastasis (mainly in lungs) and only 30% of patients with metastatic osteosarcoma achieve a 5-year event-free survival. For these reasons, identify new biomarkers for preventive diagnosis and personalized medicine is necessary. For instance, RNA sequencing (RNA-Seq) is a tool used to identify expression profiles which could be used to detect activated pathways in physiologic and pathologic conditions. Recently, voltage-gated sodium channels (Na_vs) subunits have been identified in different types of cancer. Its function has been associated with metastatic capabilities. For example, silencing of a neonatal variant of $Na_v1.5$ decreased migration in MDA-MB-231 human breast cancer cells. Additionally, Na_vs-β subunits have been related to expression regulation of Na_vs, migration, and invasion in cancer cell lines. In 2016, β4 expression was related with a metastatic phenotype. Due to the above, β4 level expression were suggested as a prognostic marker for epithelial cancer. Furthermore, expression of β1 and β2 subunits have been related to metastasis in prostate and breast cancer cell lines.

In this work, we are interested in identify the role of Na_vs in osteosarcoma metastasis process. We used RNA-Seq to identify the pattern expression of hFOB1.19 (osteoblasts cell line), SAOS-2 and SJSA-1 (osteosarcoma cell lines). Differential expression analysis allowed to identify deregulation in pathways related with extracellular matrix organization and degradation, cell-cell communication and collagen degradation, which are highly related to cancer metastasis. Additionally, $Na_v1.7$ was identified overexpressed in SJSA-1, an osteosarcoma cell line with higher metastatic capabilities than SAOS-2, and its expression was confirmed with RT-qPCR and electrophysiological analysis. On the other hand, β subunits were not detected differentially expressed in any cell line by RNA-Seq. In contrast, with RT-qPCR we could identify β1-c and β2 overexpressed in SJSA-1. After all, known if the Na_vs subunits involved have participation in osteosarcoma metastasis, through migration and invasion assays, would be important to propose a molecular mechanism and molecular biomarkers for early diagnosis.

Role of Transforming Growth Factor Beta in Neuroblastoma Bone Metastasis

Patricia Juárez*, Liria M Fajardo-Yamamoto, Brenda I Duarte, Pierrick GJ Fournier
Centro de Investigación Científica y de Educación Superior de Ensenada,

Departamento de Innovación Biomédica. Carretera Ensenada-Tijuana No. 3918,
Zona Playitas, C.P. 22860, Ensenada, B.C. México.
*Corresponding author email – pjuarez@cicese.mx

Neuroblastoma (NB) is the most common extracranial solid tumor among pediatric cancers. A unique feature of NB is the high metastatic rate, where bone is one of the main sites of metastatic occurrence. Patients with NB usually develop osteolytic lesions such as the ones that in patients with breast and prostate cancer.

Tumor cells in bone promote bone resorption and the release of growth factors from the bone matrix such as the transforming growth factor beta (TGF-beta). TGFbeta levels are increased in the bone microenvironment altering bone remodeling, promoting tumor growth, and bone destruction at the metastatic site. TGF-beta increases tumor production of pro-osteolytic factors and metastatic genes, such as PTHrP, CXCR4 and PMEPA1.

TGF-beta plays a main role in the establishment and progression of bone metastases from breast and prostate cancer and melanoma. Here, we hypothesize that TGF-beta also promotes bone metastasis from neuroblastoma. To test that we performed cellular biology experiments and we are working on the establishment of an *in vivo* model of NB bone metastases.

First, we analyzed the basal expression of TGF-beta receptors I, II y III in Neuro-2a and SK-N-AS cell lines (mouse and human NB cells lines, respectively) by qRTPCR. Both cell lines express similar levels of mRNA of the TGF-β receptors. Then, we treated the cells with TGF-beta (5 ng/ml) and evaluated the mRNA expression of TGF-beta responsive genes involved in bone metastasis. PMEPA1, PTHrP and CXCR4 (TGF-beta target genes) were slightly modulated by TGF-beta in NB cells compared to control prostate cancer cells. TGF-beta treatment did not have any effect on the proliferation and migration of NB cells, however treatment with the soluble portion of the TGF-beta receptor III (also known as soluble Betaglycan) significantly decreased their proliferation rate.

Furthermore, we are currently working on the establishment of an *in vivo* model of NB bone metastasis. Four weeks old Balb/c and CD1 Nu/Nu female mice received an intracardiac or intratibial inoculation of mouse (Neuro-2a) and human (SK-NAS) NB cells, respectively, to cause the development of bone metastasis. Further studies include the analysis by X-ray and histology of the bones of the experimental mice. Bone metastasis is a frequent complication of NB, our aim is to reach a better understanding of this devastating disease and test new treatments.

Tomosyn functions as a PKC□-regulated fusion clamp in mast cell degranulation

Iris K. Madera-Salcedo[1,2,3], Luca Danelli1,2,3, Neeraj Tiwari[1,2,3], Bárbara Dema[1,2,3], Emeline Pacreau[1,2,3], Shamila Vibhushan[1,2,3], Joëlle Birnbaum4, Chantal Agabriel5, Valérie Liabeuf4, Caroline Klingebiel6, Gaël Menasche[7,8], Marina Macias-Silva[9], Marc Benhamou[1,2,3], Nicolas Charles[1,2,3], Claudia González-Espinosa[10], Joana Vitte[11,12,13] and Ulrich Blank[1,2,3],*

[1]INSERM U1149, Centre de Recherche sur l'Inflammation, Paris, France; [2]CNRS ERL8252, Paris, France; [3]Université Paris Diderot, Sorbonne Paris Cité, Faculté de Médecine, Site Xavier Bichat, Paris, France, Inflamex Laboratory of Excellence, Paris, France; [4]Aix-Marseille University, APHM, Service de Dermatologie, Hôpital Timone, Marseille, France; [5]Aix-Marseille University, APHM, Service de Pédiatrie Multidisciplinaire, Hôpital Timone, Marseille, France; [6]Laboratoire BIOPLUS, Marseille; [7]INSERM UMR1163, Laboratory of Normal and Pathological Homeostasis of the Immune System, F-75015 Paris France; [8]Paris Descartes University-Sorbonne Paris Cité, Imagine Institute, F-75015, Paris, France; [9]Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, México; [10]Departamento de Farmacobiología, Cinvestav, México; [11]Aix-Marseille University, APHM, Laboratoire d'Immunologie, IHU Méditerranée Infection;[12]INSERM UMR 1067, Marseille, France; [13]Aix-Marseille University, CNRS UMR 7333, Marseille, France. *email: ulrich.blank@inserm.fr

Tomosyn is a Syntaxin (Stx)-binding protein that sequesters Q-SNAREs (N-ethylmaleimide-sensitive factor attachment protein receptors) inhibiting ternary SNARE complex formation and thereby cell exocytosis. Mast cells (MCs) degranulation requires Q-SNAREs and R-SNAREs to form a complex which drives the fusion of secretory granules with the plasma membrane. Is unknown whether MCs express tomosyn and whether it plays a role in the release of proinflammatory mediators induced by allergic stimulation. Our data indicates that tomosyn is expressed constitutively in MCs with a cytoplasmic distribution and negatively regulates Fc□RI induced degranulation. After Fc□RI stimulation, tomosyn was phosphorylated on serine and threonine residues and dissociated from the Q-SNARE protein syntaxin 4 (STX4), followed by its association with syntaxin 3 (STX3). Protein kinase A (PKA) and protein kinase C (PKC) inhibition prevented these activities. We identified PKC□ as the major kinase required for tomosyn threonine phosphorylation and for the regulation of the interaction with STXs. In addition, incubation with high IgE concentrations induced tomosyn expression in cultured MCs and its overexpression deceased MCs degranulation induced by the Fc□RI cross-linking. In order to analyze tomosyn function in allergic patients and to correlate it with the in vitro studies, we evaluated its expression levels. Our results indicate that in basophils from allergic patients with normal total IgE serum titers tomosyn expression was lower than in patients with high IgE titers who expressed tomosyn to the same extent as non-allergic subjects.

Our findings identified tomosyn as a negative regulator of MC degranulation that required PKC□ to switch its interaction with STX partners during fusion. The IgE-mediated upregulation of tomosyn in allergic patients may represent a counter-regulatory mechanism to limit disease development.

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Genomic stability is promoted by autophagy and NR4A nuclear receptors

Muciño Hernández G and Castro Obregón S.
Department of Neurodevelopment and Physiology, Institute of Cellular Physiology, UNAM. Circuito Exterior S/N, Ciudad Universitaria, Coyoacán, 04510, Ciudad de México. Phone: (52)(55)5622-5676; e-mail: scastro@ifc.unam.mx

As a consequence of genotoxic stress, cells orchestrate molecular reactions through the whole cell in order to repair genomic alterations. We are interested in two events observed during genotoxic stress, the activation of autophagy and the recruitment of NR4A nuclear receptors to DNA lesions. On one hand, defects on autophagy or NR4A expression lead to DNA repair deficiencies. On the other hand NR4A1 promotes autophagic cell death. Based on these observations, we hypothesize that NR4A receptors promote DNA repair by modulating autophagy. We stablished a DNA double strand breaks and repair model in mouse embryonic fibroblast and A549 cancerous cells. During DNA damage, both cell types showed a nuclear recruitment of NR4A1 and NR4A3 with a similar intranuclear distribution than the DNA damage marker YH2AX. Simultaneously, we observed an induction of autophagy, starting since the DNA damage induction and persisting along the DNA repair process. As a result of genotoxic treatment in fibroblast, we detected in the cytoplasm several nuclear components including DNA damaged associated with proteins involved in the autophagic process, suggesting an autophagic degradation of nuclear damaged material. Interestingly, when autophagy was induced previous to DNA damage in fibroblast, there was a reduction in DNA breaks. Intriguingly, the opposite occurred in A549 cells, which have a higher basal autophagy. We found that an induction of autophagy before DNA damage worsen DNA double strand breaks. All together our results highlight the contribution of autophagy to maintain genome integrity. How the nuclear orphan receptors NR4A1 and NR4A3 could be modulating nuclear and DNA stability will be discussed.

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"Characterization of the function of the *non-coding intermediate RNA ImncEIN2* in the ethylene perception pathway".

Jesús Nieto Hernandez, Jaime Aportela Cortez[1], Sergio Casas Flores[2], Catalina Arenas Huertero[1,3]. Posgrado en Bioprocesos, Facultad de Ciencias Químicas[1], Universidad Autónoma de San Luis Potosí. Instituto Potosino de Investigación en Ciencia y Tecnología[2]. Facultad de Ciencias, Universidad Autónoma de San Luis Potosí[3]. Diagonal Salvador Nava Mtz s/n, Zona Universitaria, San Luis Potosí., SLP México. CP 78290. catalina.arenas@uaslp.mx

According to the development of genomics, bioinformatics and several applications of high-throughput sequencing technology, more transcriptional units have been discovered with little or no protein coding potential. These RNA molecules are called non-coding RNA *(ncRNA).* " The non-coding RNAs can be divided arbitrarily into three classes according to their length. The ncRNA with a length less than 50 nucleotides (nt), are known as small RNAs. Those with a length equal to or greater than 50nt and less than 300nt are known as intermediate non-coding RNAs *(imncRNAs)* and those with a length equal to or greater than 300nt are known as long non-coding RNAs *(lncRNA)*1.

In the model plant *Arabidopsis thaliana*, there is very limited information of the new ImncRNAs. Some features of the *ImncRNAs* presents an average abundance like the mRNA in the seedlings. In addition, *ImncRNAs* are regulated by genetic and epigenetic mechanisms similar to those of genes that encode proteins, and some showed expression patterns regulated by the development and down-regulation of several *ImncRNAs* resulting in molecular changes and obvious phenotypes of development abnormal, indicating a functional importance of *ImncRNAs* in the growth and development of plants. For all these characteristics, the *ImncRNAs* are considered as independent functional and regulatory components, distinct from the long non-coding RNAs.2

In plants, the perception of ethylene occurs in the membrane of the endoplasmic reticulum and signal transduction leads to the activation of a transcriptional cascade that hints to the activation of various genes that respond to ethylene. In this route, the *EIN2* protein, located in the endoplasmic reticulum, plays a central role in the transmission of the signal in the perception of ethylene from the endoplasmic reticulum to the nucleus3,4. In this work, propose the characterization of the *noncoding intermediate EIN2 (ImncEIN2),* in the *A. thaliana* model. We hypothesize that ImncEIN*2* could play an important role in the ethylene perception and response pathway by regulating the activity of the Ethylene Insensitive 2 protein (*EIN2)*.

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Natural intra-species variation of the mutational effects of the TOR pathway in cell survivorship

J. Abraham Avelar-Rivas, Alexander De Luna

Unidad de Genómica Avanzada (Langebio), Centro de Investigación y de Estudios Avanzados del IPN
Irapuato, Gto. México, CP 36824, +52 (462) 166 3000 ext. 3069 jesus.avelar@cinvestav.mx, alexander.deluna@cinvestav.mx

Inactivation of the TOR pathway increases cell survivorship and the lifespan of organisms from yeast to mammals. However, little is known about how intra-species natural genetic variation shapes this effect within a single species. In the budding yeast, cellular aging can be modeled by measuring its chronological lifespan, namely the time that a non-dividing population of cells survives in stationary phase. This model has allowed the identification of genes and processes later proven to influence aging in other organisms, including humans. Here, we explore the way in which chronological lifespan is affected upon deletion of genes of the TOR/Sch9 and RAS/PKA pathways in six strains of *Saccharomyces cerevisiae* isolated from different niches in Europe and the Americas. Specifically, we measured in each case the relative lifespan of twelve gene-deletion strains tagged with RFP in co-culture with their corresponding YFP-tagged wild type; the change in the proportion of fluorescent signals throughout the experiment was used to determine the relative survivorship of each mutant in each genetic background. Our results show widespread variation in both sign and magnitude of the lifespan effect, indicating that the genetic background is an important determinant of cell survivorship. Importantly, we found that there are at least two patterns of variation in which either all of the independent deletions of signaling genes increase or decrease lifespan. Furthermore, we observed that strains with no lifespan extension are irresponsive to calorie restriction, another conserved treatment that usually increases lifespan through TOR signaling. To better understand the observed phenotypic variation, we tested whether changes in mutational effects are explained by variation within the same pathway, specifically by measuring genetic interactions of the signaling genes with *RIM15*, the main integrator of signals regulating transcription factors downstream of TOR. Together, our results suggest that intra-species genetic variation may block or even reverse the beneficial effect of TOR inactivation by intricate intra- and inter-pathway cellular crosstalks.

Epikouros: Empowering society with scientific data

Gabriel Del Río, David Yao, Jerónimo Vargas
Instituto de Fisiología Celular, UNAM; Circuito exterior s/n, CU, UNAM, CDMX.
gdelrio@ifc.unam.mx.

The XXI century is the era of data and many activists around the globe have risked their lives to promote the freedom of data. In this realm, science should not be an exemption, specially considering that scientific knowledge should be publicly available to promote sustainability and equity in the world [1]. Despite different efforts to achieve open access to scientific data, much remains to be done [2]. The current flow to generate and communicate scientific data involves governments and private initiative that economically support scientists to generate data; then this data is publicized through an editorial that economically charges scientists for making their data available and it demands the copyrights for itself, the costs of that publications is commonly transferred to governments and private initiative; finally, editorials economically charge readers (scientists and members of the society in general) to provide access to scientific data. Hence, these economic and knowledge flows that society use to promote science gets concentrated into editorials, which do not retribute science as much. Similar problems where concentrating the resources into a common pile end up affecting the providers and receivers of the resources is common in many other disciplines (*e.g.*, banks, governments, etc). A modern technical solution for such problems, blockchains, has been evaluated in economics under the concept of crypto-coins (*e.g.*, bitcoin); the main features of blockchains are their transparency and security in transactions [3]. We are developing a novel implementation of a blockchain where transactions are promoted by those with new scientific data (proof of importance), hence combining for the first time the economic model with the security of transactions into the blockchain; this platform is powered by machine-learning techniques and other IT solutions. The aim of this technological development is to create a decentralized editorial platform, Epikouros, to redirect the economic and knowledge flows back to science and society. To achieve this, scientists publishing through the Epikouros platform will not pay for publishing, instead they will receive payments in the form of a crypto-currency; as any other economic system, the more the coin is used, the more its value. Hence, Epikouros will for the first time provide a value to scientific data to empower scientists and return scientific data back to the society.

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Testing the Domino Theory of Gene Loss in *Buchnera aphidicola*: The Relevance of Epistatic Interactions

Luis Delaye, Gil Bor, Andrés Moya, David J. Martínez-Cano

CINVESTAV Irapuato
Km. 9.6 Libramiento Norte Carr. Irapuato-León 36824
Irapuato Gto. México
Tel: +52 (462) 6239669
E-mail: luis.delaye@cinvestav.mx

The domino theory of gene loss states that when some particular gene loses its function and cripples a cellular function, selection will relax in all functionally related genes, which may allow for the non-functionalization and loss of these genes. Here we study the role of epistasis in determining the pattern of gene losses in a set of genes participating in cell envelope biogenesis in the endosymbiotic bacteria *Buchnera aphidicola*. We provide statistical evidence indicating pairs of genes in *B. aphidicola* showing correlated gene loss tend to have orthologs in *Escherichia coli* known to have alleviating epistasis. In contrast, pairs of genes in *B. aphidicola* not showing correlated gene loss tend to have orthologs in *E. coli* known to have aggravating epistasis. These results suggest that during the process of genome reduction in *B. aphidicola* by gene loss, positive or alleviating epistasis facilitates correlated gene losses while negative or aggravating epistasis impairs correlated gene losses. We interpret this as evidence that the reduced proteome of *B. aphidicola* contains less pathway redundancy and more compensatory interactions, mimicking the situation of *E. coli* when grown under environmental constrains.

Experimental evolution of resistance to antibiotics under different intensities of selective pressure.

Sandra Mayoral Álvarez, Rafael Peña Miller and Ayari Fuentes Hernández*.
Laboratorio de biología de sistemas y biología sintética, Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México, Av. Universidad s/n, Chamilpa, 62210 Cuernavaca, Morelos.
*ayarifh@ccg.unam.mx

Most of our understanding of drug resistance evolution is based on wet-lab experiments performed in well-mixed environments with controlled environmental conditions. But natural environments are heterogeneous, both in time and space, and expose bacterial populations to a dynamic range of antimicrobial concentrations. In this presentation we are interested in addressing the following question: ¿how does the adaptive landscape changes as a function of the intensity of the selective pressure? In particular, we are interested in using experimental evolution to compare bacterial isolates with the same phenotype (level of drug resistance), but achieved through diverging evolutionary histories (previous exposure to drugs).

Materials and methods: We use Escherichia coli MG1655 and a beta-lactam antibiotic, ampicillin. Our experimental protocol is based on evolutionary experiments where an isogenic bacterial population is evolving under different intensity in the selection pressure: high and mild selection. First, we determine the Minimal Inhibitory Concentration (MIC) of wild-type strain with a dose-response experiment in minimal media (M9), using 96-well microplates and a plate reader (BioTeK EL808x) to measure bacterial density. We selected two bacterial populations exposed to different antibiotic concentrations, for the mild selection regime we sample from a population that achieves 50% inhibition (IC50) and for the high selection regimen we transfer into fresh media a population presenting 90% growth inhibition (IC90) and every day inoculated a new dose response assay. Finally, for both regimes, different isolates were transferred to a drug-free environment to evaluate the correlation between drug resistance and the associated fitness cost with the intensity of the selective pressure used during the evolution experiment.

Results and conclusions: We observed that the adaptation of bacterial populations to the new environment can be affected by the selective pressure intensity. Bacterial populations under both regimes reached the same level of resistance (10 MIC,compared with the wild-type strain), but strong selective pressure had a faster rate of adaptation in contrast with the medium selective pressure. However, the data suggest that fitness cost might not be affected by acquired resistance due to compensatory mutations.

IsomiR-index: a method for analyzing quantitatively the isomiR landscape

O. Omar Moreno-Proo, Mariana Flores-Torres, Filiberto Bautista-Moreno, S. Eréndira Avendaño Vázquez, C. Fabián Flores-Jasso.
Instituto Nacional de Medicina Genómica
C.P. 14610 Teléfono. +52 (55) 5350-1900 ext. 1138 cfflores@inmegen.gob.mx

microRNAs (miRNAs) are a class of small RNA that participate in the regulation of gene expression at the posttranscriptional level. With the use of deep sequencing methods, isoforms belonging to genes of miRNAs have been identified; these are called isomiRs. IsomiRs are miRNAs that contain variations with respect to the sequence that has been reported as *canonical* in a species. These variations constitute additions, substitutions or deletions of one or more nucleotides from the canonical sequence. Although the presence of isomiRs is known, many studies have yet to quantitatively describe the complete profile of these isoforms in different biological samples. It is unclear hitherto to what extent the isomiRs contribute to gene expression regulation primarily because there is not graphic or statistical method for understanding isomiR variation as a whole. By understanding this variation it might be possible that by revealing the proportions of isomiRs populations we can make an inference about their origin and role in the miRNA pathway. For this study, we developed a method to visualize and compare the complete isomiR landscape in different biological samples. For the complete profile analysis of miRNAs and isomiRs present in different biological samples, we used public databases that offered raw data from libraries from deep sequencing assays directed to identify miRNAs. In order to build a isomiR index we grouped the microRNA sequences by their abundance in each library and identified the *Most Abundant Sequence* (MAS) as the sequence that turned out to be the most abundant recorded for each miRNA. The "isomiRs" are represented by the sum of the number of all variants other than the MAS sequence for each miRNA. We find that in the majority of the samples analyzed miRNAs are preferentially distributed towards their MAS sequences, regardless of the abundance that each miRNA represents in each particular library. However, there are examples of highly abundant miRNAs in some libraries that are preferentially shown in their isomiRs forms. We compared libraries from exosomes and cell lines and observed a correlation in the presence of MAS miRNAs in both samples. On the other hand there is also a proportion of miRNAs (close to 40%) that does not show this behavior, as there is no defined distribution for their MAS or isomiRs forms and some of them are not shared among the samples. This finding reveals that for some microRNAs, the abundance of isomiRs is greater than the abundance of the MAS of microRNAs, sometimes referred to as canonical. We also analyzed libraries from cells lacking Dicer —the enzyme that transforms pre-microRNAs into their mature forms, and found that most microRNAs are represented as isomiRs, suggesting a mechanism for their biogenesis. Finally, we performed the same type of analysis in samples prepared from exosome-contained and exosome-free microRNAs and find that by using the isomiR index it is possible to asses whether an exosome sample is reasonably clean from contaminant proteins. In conclusion, isomiR index can facilitate the research of microRNAs variation within and across samples.

De novo assembly of small RNA sequencing reads improves species assignment in combined parasite-host samples

Obed Ramírez-Sánchez, Cei Abreu-Goodger
Unidad de Genómica Avanzada (Langebio), Cinvestav, Km 9.6 Libramiento Norte Carretera Irapuato-León, Irapuato, GTO, México.
tel: +52 462 166 3000, email: {obed.ramirez,cei.abreu}@cinvestav.mx

Introduction: microRNAs are small RNAs involved in different biological processes such as development, defense and disease. Recently, small RNA-mediated communication between parasites and hosts has been described for several species [1-3]. During infection, parasites secrete small RNAs that can be internalized by host cells, with many functional implications [1-3].

Problem: Current technology allows massive sequencing of small RNAs sampled from parasites within their hosts. However, it is hard to identify the origin of many RNAs since they can align equally well to both genomes because of small read lengths and high levels of conservation of some microRNAs, and tRNA or rRNA fragments [4]. If these ambiguous reads are discarded, information is lost and cannot be used during further analysis (*e.g.* differential expression or target prediction).

Results: We worked with small RNA sequencing data from three parasite-host systems: a) *Botrytis cinerea – Arabidopsis thaliana,* b) *Cuscuta campestris –Arabidopsis thaliana,* and c) *Heligmosomoides bakeri – Mus musculus*. For these, we found that 10.2%, 43.9% and 11.3% of the reads ambiguously mapped to both genomes. Longer reads tend to map correctly to parasite or host, but in the typical length of miRNAs (21-24 nt) around 8% of reads are ambiguous. To extend the length of sequenced reads, we evaluated six *de novo* assembly programs (Tadpole, Trinity, rnaSpades, SOAPdenovo-Trans, TransAbyss y Oases) and a range of parameters. The best assemblies where obtained using Trinity, followed by Tadpole and rnaSpades. *De novo* assembly drastically reduced ambiguous reads to 0.2%, 12.9% and 0.5%.

Conclusion: *De novo* assembly using Trinity allowed us to assign most sequenced small RNAs reads to the correct organism from which they originated. Other applications of this work include taxonomic assignment (in metatranscriptomes or contaminant identification) and differential expression analysis.

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Inquiry Based Science Education (IBSE) as a tool for learning Molecular Biology. The experience in México.

Viviana Escobar-Sánchez[1], James Karkashian Córdoba[2], Alicia González Manjarrez[3], Claudia Segal-Kischinevzky[1].
vv.escobar@ciencias.unam.mx / claudiasegal@ciencias.unam.mx
[1]*Departamento de Biología Celular. Facultad de Ciencias.*
[2] *Universidad de Costa Rica, Escuela de Biología*
[3]*Departamento de Genética Molecular, Instituto de Fisiología Celular*
UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO. Cd. Mx. C.P. 04510, MÉXICO.

In Mexico, only 44 of the population between 15 and 24 years currently attends school (2015, INEGI). Regrettably, less than 10% of the young people that enroll in university studies apply for a scientific career. Because of this, promoting interest in scientific research becomes an important undertaking. The National Autonomous University of Mexico (UNAM) has a great interest in reinforcing the teaching learning process at high school level, making great institutional efforts to update their teachers.

The challenge is huge. In order to address this issue, we are participating in a Latin American Project of portable laboratories. Thanks to a grant provided by the Wellcome Trust through a draft submitted by the Red Latinoamericana de Biología (RELAB), we acquired a Molecular Biology portable laboratory in order to make available exciting laboratory experiences to high school students. Amongst all the experimental activities, students can identify the culprit through the analysis of the DNA from suspects of a crime scene, perform the extraction and amplification of their own DNA for genotyping, use the green fluorescent protein from a jellyfish for the genetic modification of bacteria, perform gel electrophoresis.

We have already taught seven courses to high school teachers, who have been very excited and interested in developing the experimental activities in their own teaching places. The portable laboratory has been successfully implemented in eight high schools of our university, involving around 700 teenagers.

Teachers and high school students who have become part of this experience, refer to it as an activity that significantly affects the teaching-learning process and motivates them to deepen on these topics.

In our experience, good intentions, equipment, inputs and strategy are definitely not enough. The motivation of the managers from each campus is completely necessary and encourages the teachers to leave their own comfortable zone to include IBSE in their teaching practice and commit themselves to benefit their students: young students of the baccalaureate system.

This project has a multiplier effect, since we have both the equipment and the human resources: the teachers who have already taken the courses. We consider that encouraging young people to adopt the scientific method will provide them with better tools to face the challenges they will meet, regardless of the study area in which they specialize.

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Design of artificial virus-like protein-DNA nanoparticles with programmable self-assembly

Carlos Calcines Cruz, Javier Fernández Segura, Itzel González Carmona, Lucila Arroyo Rabasa, Armando Hernández García.
Instituto de Química, UNAM. Circuito Exterior, Ciudad Universitaria, Delegación Coyoacán C.P. 04510, Cd. de Mx. armandohg@iquimica.unam.mx.

Current viromimetic research focuses on the identification of *de novo* capsomers that assemble as artificial virus-like particles which reproduce certain viral features: homogeneity, cooperative self-assembly and DNA or RNA encapsidation. However, little attention has been payed to the recognition by the artificial capsomers of specific DNA or RNA molecules, such as those coding for the capsomers themselves. The latter has proved critical for the viral multiplication cycle, for instance, in retroviruses. The assembly of retrovirus capsomers onto the viral RNA relies on the recognition of a nucleotide sequence, known as the encapsidation signal, by a nucleocapsid protein. Once this initial binding reaction (K_D~50-100 nM) occurs more capsomers are recruited cooperatively to the nucleic acid template until encapsidation is complete. The *in vitro* recreation of the previous scenario is the aim of this work, using *de novo* designed and chimeric proteins.

For the recognition of the nucleotide sequence (*i.e.* the encapsidation signal) and the recruitment of capsomers we used a chimeric protein consisting of a catalytically inactivated endonuclease from the bacterial CRISPR-Cas system fused to a dimerizing protein: either the rapamycin binding domain of human mTOR (FKBP) or the FKBP-rapamycin binding protein (FRB). In the presence of rapamycin, FKBP and FRB bind with a K_D~10 nM. Once the endonuclease fused to either FKBP or FRB has bound to the encapsidation signal, and in the presence of rapamycin, it can recruit another chimeric protein consisting of either FRB or FKBP fused to a self-assembly domain derived from the silk protein. This self-assembly domain will, in turn, recruit the capsomers consisting of the self-assembly domain itself fused to a lysine tail for electrostatic and cooperative binding with DNA (K_D~730 nM, measured by Bio-Layer Interferometry). Such a system is expected to elicit a sequence-specific encapsidation along a template DNA or RNA, as observed in retroviruses.

So far, all chimeric proteins have been obtained through standard cloning methods, expressed and some have been purified. Proteins consisting of the self-assembly domain fused to either the lysine tail, FKBP or FRB were expressed in *Pichia pastoris* and purified through salt and acetone precipitation. The inactivated endonucleases (dCas9 and dCas12a) fused to either FKBP or FRB were expressed in *Escherichia coli* and are being purified by column chromatography. Following this stage, we will study the assemblies by electrophoresis mobility shift assay, dynamic light scattering and atomic force microscopy.

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Study of phenolic compounds present in tequila vinasse for its recovery and integral use on biorefinery procesess.

Cornejo-Corona Ivette*, Orona-Tamayo Domancar, Ramírez-Hernández Christian Eduardo, Rodríguez-Rodríguez, Stephanie Maviel & Guerrero-Barranco Arturo.

Centro de Innovación Aplicada en Tecnologías Competitivas
Omega 201, Col. Industrial Delta CP. 37545 León, Guanajuato
Tel. +52(477)710 00 11
*icornejo@ciatec.mx

The tequila industry generates two main residues in its process derived from agave, one of them is the solid part called bagasse and the other is the liquid part obtained from the distiller known as vinasse. The vinasse in particular is produced in a ratio of about 10 to 12 liters per liter of tequila, has a high organic content that causes damage to ecosystems by anoxia and water acidification. Mexico produces more than 170 million liters of tequila annually which generates around 2295 million liters of vinasse which represents a serious environmental problem. High concentrations of phenols (0.6 g/l) have been found, and it that have not been characterized but are cause of acidity and coloring of the vinasses. In another hand, some kind phenols are in high demand in the pharmaceutical industry as antioxidants, sanitizers, antibacterials, as flame retardants and even in the leather industry. This project characterized the phenolic compounds using colorimetric methods of detection of antioxidant activity and an ultra-high performance liquid chromatography (UHPLC) with UV detection method was developed for the fast quantitation of the most represented and biologically important vinasse compounds. Our analysis showed that the most abundant compound with over 37 mg/100g is catechin which is a powerful antioxidant and commonly used as a nutraceutical.

Several studies have demonstrated the stability of catechins at temperatures up to 120°C in green tea extracts, they are known to have superoxide dismutase activity also antibiotic effect with disruptive effect during the bacterial DNA replication process has also been demonstrated.

Until now the vinasse has been treated and disposed of as a waste, however we have demonstrated the presence of high value compounds that can be recovered, which will allow the tequila industry to adapt its process and become part of the biorefinery system.

New source of natural bioactive compounds: Phenolic compounds and melanin of residues from energetic plant biomass with a high potential of nutraceutics

Domancar Orona Tamayo[*1], *Elizabeth Quintana Rodríguez*[1], *Christian Eduardo Ramírez-Hernández*[1], *Ivette Cornejo Corona*[1], *José de Jesús Esparza Claudio*[1], *María Elena Valverde*[2] & *Octavio Paredes López*[2].
[1]Departamento de Soluciones Tecnológicas. Centro de Innovación en Tecnologías Competitivas (CIATEC, A.C.). Omega #201 Col. Industrial Delta. León, Gto.
[2]Departamento de Biotecnología y Bioquímica. CINVESTAV-Irapuato. Km 9.6. Lib Nte. 36821 Irapuato, Gto
***dorona@ciatec.mx; domancar@gmail.com**

Due to severe energy crisis, alternative energy sources are obtaining importance. Nowadays, the concept of biodiesel production derived from energy plants has widespread attention in this globalized world. *Jatropha curcas* and *Ricinus communis* are energetic plants from the Euphorbiaceae family, produced a high oil-seed that commonly used in the production of biodiesel by transterification. *J. curcas* and *R. communis* seeds contain about 30-35%[1] and 46-55%[2] of oil, respectively, very useful as feedstock to biodiesel production. However, during seed processing to the oil extraction there is generated tons of waste production. Has been estimated with every ton of *Jatropha* and *R. communis* oil, they produced approximately 3 tons of waste material[3] in form of outer shells, hulls and seed cake. The crammed residues produce the settlement of nests of rodents that are vectors of diseases for humans and also cause ecological problems over the decomposition stage. The shells, hulls and seed cakes of these plants contain different bioactive compounds such as polyphenols[4]. Polyphenols are strong antioxidants compounds and are excellent to scavenge free-radical and to chelate metal ions[5]. In another hand, the melanin is a compound that some plants contain in their structures with a broad spectrum of biological activities, including protecting against UV-radiation, enzymatic lysis, oxidants, and even to chelating metal ions[5]. An interesting possibility of utilization plant residues could be as a strong antioxidant and photoprotective solution with a different application in pharmaceutic and food industries[6]. The aim of this work was to obtain and evaluate the *in vitro* antioxidant and antihypertensive activities of polyphenols from shells, hulls and seed cake. In addition, the isolation and characterization of melanin pigment from the hulls and evaluation of antioxidant properties. As expected, polyphenols have a strong antioxidant activity, and we identified by UHPLC different phenols and flavonoids concentrations such as protocatecuic, rosmarinic, galic and chlorogenic acids contents between plant residues. In sum, these materials may be considered as functional ingredients with a high antioxidant potential in foods and cosmetics with a features may provide effective outstanding benefits for the human health such as anti-carcinogenic, antioxidant and photoprotective effects for the skin and cells[6]

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Metabolic engineering to improve hydrogen production by *Escherichia coli*

[1]Victor E. Balderas-Hernandez, [1]Kathya P. Landeros Maldonado, [2]Adam Smoliński and [1]Antonio De Leon Rodriguez*

[1]Instituto Potosino de Investigacion Cientifica y Tecnologica, A.C. Camino a la Presa San Jose 2055. Lomas 4a Sec, San Luis Potosi, S.L.P. 78216, Mexico. [2]Central Mining Institute, Pl. Gwarków 1, 40-166 Katowice, Poland. * E-mail: aleonr@ipicyt.edu.mx, aleonr@me.com

Abstract

Biological production of hydrogen (H_2) from lignocellulosic biomass composed by pentoses and hexoses offers social, economic and energetic benefits. However, a critical issue for the use of bacteria, such as *Escherichia coli*, as biocatalyst for the H_2 production is the non-efficient consumption of mixed carbohydrates-substrates as carbon source because the carbon catabolite repression (CCR), a regulatory mechanism that causes sequential utilization of carbohydrates and in some cases null consumption of less preferred sugars, such as the xylose. In *E. coli*, the phosphotransferase system (PTS) is a key component in the CCR regulation. In this work, we evaluate the effects of elimination of PTS genes such as *crr* and *ptsG*, as well as the modification of genes involved in the central carbon metabolism on consumption of glucose-xylose mixtures by *E. coli* WDH (Δ*hycA*) strain and its concomitant H_2 production. *E. coli* WDH strain produced 2,000 ml/l of H_2 and consumed all the glucose, however xylose remained unconsumed from a mixture of glucose-xylose of 10 g each. Elimination of glucose-specific enzyme IIA (*crr*) caused a detrimental effect on the growth, carbohydrates consumption and H_2 production. In contrast, elimination of glucose permease-enzyme IIB (*ptsG*) caused a 30% increment on the consumption of xylose, the simultaneously consumption of glucose/xylose, and a 22% increment on the cumulative H_2 production was observed in comparison with the parental WDH strain. In addition, *pstG*, *frdD* (fumarate reductase) and *ldhA* (D-lactate dehydrogenase) genes were also eliminated and the resulting strain designated as *E. coli* WDH-GFA (Δ*ptsG*, Δ*frdD,* Δ*ldhA*) consumed 40% more xylose and produced 3.7-times more H_2 than the WDH strain. Besides H_2, *E. coli* WDH-GFA strain also produced ethanol as the main carbon byproduct. These results show that elimination of *ptsG*, in combination with a modified into the central carbon metabolism improve the production of hydrogen. We thank to the partial financial support by the SENER CEMIE-BIO 249564 and CONACYT- Basicas 281700.

Keywords: Biofuels, simultaneous carbohydrate consumption, carbon catabolite repression, hydrogen.

Characterization of catechol dioxygenases produced by aromatic hydrocarbons-degrading Pseudomonas strains obtained from the Gulf of México for its use in bioremediation.

Selma Julieta Rodríguez-Salazar[1], Sofía Millán-López[1], Katya Ornelas Ocampo[1], José Luis Rodríguez Mejía, Luis Felipe Muriel-Millán[1], Liliana Pardo-López[1]
[1]Instituto de Biotecnología UNAM, México
Gulf of Mexico Research Consortium, Instituto de Biotecnología, Av. Universidad 2001, Col. Chamilpa, Cuernavaca, Morelos 62210, México. Universidad Nacional Autónoma de México, julieta@ibt.unam.mx, liliana@ibt.unam.mx

Polycyclic aromatic compounds constitute an important fraction of petroleum hydrocarbons; most of the polycyclic aromatic compounds are metabolized into the molecule of catechol, a common intermediate in the aerobic degradation of aromatic compounds.

Catechol dioxygenases play a central role in degradation of aromatic compounds because they catalyse the critical and chemically difficult aromatic ring-cleavage reaction. Catechol oxidation can occur through the two ring-cleavage pathways (ortho and meta cleavage) catalysed by C12DO (catechol 1,2-dioxygenase) and C23DO (catechol 2,3-dioxygenase), respectively. Microorganisms that contain catechol dioxygenases are able to utilize aromatic molecules as their sole source of carbon and energy, not only to survive in polluted areas but in doing so, to decontaminate the soils.

The aim of our study are the cloning, expression and characterization of two catechol dioxygenases from *Pseudomonas* strains obtained from sediment and water samples of the Gulf of Mexico.

We analyzed the activities of C12DO and C23DO in 40 bacterial isolates obtained from water (between 50 and 3000 meters deep) and sediments (from 500 to 3000 meters deep) and 60 bacterial consortia obtained from oil-enriched culture as the only carbon source to enrich the population of bacteria favoring the selection of strains with dioxygenase activity. We obtained 2 samples with high activity for each enzymatic activity tested. The samples resulted to belong to the *Pseudomonadaceae* family when 16s DNA analysis was performed.

We designed specific oligonucleotides for the amplification of the genes that code for the C12 DO and C23DO from the *Pseudomonas* samples and we proceed to perform the cloning of the fragments amplified in an expression vector containing a C-terminal histidine tag to purify the enzyme. The overexpression is under the control of an IPTG inducible promoter and the transformation was performed in BL21 *E.coli* cells.

We tested C12DO enzymatic activity first in *E. coli* cells and proceed to purify the protein under native conditions to determine the correct expression, purification and activity. The next step is to carry out enzymatic assays at different conditions of temperature, pH and salinity to determine the optimal ranges from these marine dioxygenases.

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Antimicrobial activity of redclaw crayfish *Cherax quadricarinatus* Pro-rich recombinant peptide

Gisela Ruvalcaba-Rodríguez, Javier Jearim Maldonado-Romo, Lizandro René Rivera-Rangel, David Chavez-Flores, Antonio García-Triana.
Universidad Autonoma de Chihuahua, Facultad de Ciencias Químicas, circuito universitario campus II C.P.31125, +526141508161, atriana@uach.mx

Pro-rich is a short chain peptide derived from an unknown coding sequence of the carboxyl-terminal end of the Glutathione peroxidase protein found in the redclaw crayfish *Cherax quadricarinatus*, with predicted antimicrobial activity. Pro-rich was recombinantly over-expressed in *Escherichia coli* and purified for sequencing. The purified peptide (1 mg/mL) was used in qualitative sensitive tests with bacterial species of medical importance, resulting in a positive antimicrobial activity against *Klebsiella pneumoniae* and *E. coli*. Subsequently, it was subjected to MICs assays, which were carried out with 0.6mg/mL and partial purifications, in order to verify its potential in non-pure phases. Our results indicate that partially purified peptide and low concentrations generate antimicrobial activity in pathogen bacterias.

Structural studies on the tail domain of the human cytoplasmic Dynein-1

Field: Structural Biology.
Edgar Morales Ríos[1,2], Saúl Santiago Sánchez[1] and Andrew P Carter[2]
[1]Cinvestav. Lab 2 Biochemistry. Av. IPN 2508 Tel.: 57473800 ext. 5234
[2]Laboratory of Molecular Biology-MRC. Francis Crick Av. Cambridge CB2 0QH, UK

Microtubule-based cellular traffic is polarized in a minus and a plus end. Human cytoplasmic dynein-1 (dynein) is a multi-subunit motor that transports a great diversity of cargoes, including several viruses, into the minus end of the microtubules (from the membrane to the nucleus)1. In order for dynein to start its processive movement, it requires its cofactor, dynactin, and one cargo adaptor2. Dynein is a 1.4 MDa complex composed of 2 copies of 6 different subunits (HC, IC, LIC, Robl, LC8 and Tctex), dynactin is a 1.1 MDa complex, formed by 23 subunits. There is, at least, 12 different cargo adaptors, all of them able to activate the processive movement of the complex dynein-dynactin. These cargo adaptors have no sequence motifs that could relate each other. The only common feature of these adaptors, is a long coiled coil, binding site for dynein-dynactin complex.

There has been three structures of the complex dynein tail-dynactin-cargo adaptor solved by Cryo-EM, two at low resolution, with the cargo adaptors BICD2 and HOOK3, and one at mid-resolution, with the cargo adaptor BICDL1. Another interesting feature observed was that BICD2 binds one dynein to dynactin (TDB), nevertheless, BICDL1 (TDR) and HOOK3 (TDH) bind two dyneins to dynactin. TDB complex has approximately half of the force and speed compared to TDR and TDH. Taking these results together, it is proposed that dynactin works as a natural scaffold to bind one or two dyneins, thus the number of dyneins will be controlled by the cargo adaptor.

The resolution reached with cryo-EM is not enough to establish an atomic model of how the cargo adaptor binds to dynein and dynactin, because the intrinsic limits of the technique but most importantly, because of the high movement of the tail domain of dynein.

With this project we are proposing to obtain atomic structures from these proteins and establish a model of how dynein binds to the cargo adaptors. To reach this aim, we are purifying and crystallizing several fragments of the HC of dynein expressed in *E. coli* in order. We have now purified 35 different fragments of the N-Terminus of the HC of dynein by IMAC and gel filtration. From these fragments, we have induced crystals from a 64 kDa fragment, the resolution of the diffraction of these crystals is 5.2 Å. Also, we are screening the binding of this fragment with several viral proteins, by finding a viral proteins that binds to the HC, and in collaboration with Dr. Andrew P Carter, we will solve the cryo-EM structure of the complex dynein-dynactin-viral protein.

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Structural and thermodynamic contribution of the binding site residues in the lysine-arginine-ornithine binding protein (LAO) to ligand affinity and selectivity.

Jesús Renán Vergara Gutiérrez; Sergio Romero Romero; Isabel Velázquez López; Adela Rodríguez, Alejandro Sosa Peinado; Daniel Alejandro Fernández Velasco.
Laboratorio de Fisicoquímica e Ingeniería de Proteínas, Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad Universitaria. Ciudad Universitaria, 04510, Ciudad de México, México. Phone number: +52 5556232259. E-mail: renanvg05@gmail.com (JRVG), fdaniel@unam.mx (DAFV)

Molecular recognition is the cornerstone for all cellular processes and biotechnology, however, the rational design of protein-ligand interactions has been extremely unsuccessful. Such difficulties indicate that is necessary to study in detail the ligand binding process in natural systems, especially the binding energetics and its structural relationship. For this reason, we studied the contribution of all the binding site residues of the lysine-arginine-ornithine binding protein (LAO) to the affinity and selectivity for arginine and histidine. Both ligands contact the same protein residues but present different affinities (Kd = 1nM and 2.7 □M, respectively). The LAO binding site consists of residues D161, R77, S72 (main chain binding group), F52 and Y14 (side chain binding group) which form common interactions with both ligands, as well as D11 and S69 (guanidine binding group) that interact only with the arginine side chain. Residues D30 and S70 stabilize a water molecule that makes contact exclusively with arginine. To determine the thermodynamic and structural role of these residues in ligand binding, we characterized single alanine mutants by isothermal titration calorimetry (ITC) and obtained crystallographic structures. D161A and R77A mutants have an important impact in the affinity for both arginine and histidine ($\Delta\Delta G_{wt\text{-}mut}$ = 5 kcal/mol), however, the thermodynamic effect depends on the ligand as a result of the differential incorporation of water molecules to the binding site. Mutation F52A has the higher effect in binding affinity, whereas Y14A presents a considerable but lower impact; in both cases arginine binding is more affected than histidine binding. Surprisingly, mutant S69A shows no measurable impact on affinity; whereas D11A shows a difference in the effect for arginine and histidine binding of only 1.3 kcal/mol. Finally, D30A and S70A mutants have a very similar thermodynamic and structural effect, both affect arginine binding more than histidine binding. In summary, by means of a correlation between the thermodynamic and structural effects of removing protein-ligand contacts, we have been able to determine the contribution of each residue to ligand binding, . Our results show that D161, R77 and F52 are the most relevant residues for binding and, more important, D11 is not the main responsible of the affinity difference between ligands, as previously suggested when the structure of LAO was solved. We conclude that structural information is not enough to determine the contribution of protein-ligand interaction to affinity and selectivity and more experimental studies are needed to increase the protein-ligand design success.

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Computational approach to optimize the PTP1B inhibitor JM151.

Mara Ibeth Campos Almazán, Alfredo Téllez Valencia, María Alicia Hernández Campos, Antonio Romo Mancillas, Erick Sierra Campos, Mónica Andrea Valdez Solana, Claudia Isela Avitia Domínguez.

Facultad de Medicina y Nutrición Universidad Juárez del Estado de Durango Av. Universidad s/n, Centro, C.P. 34000, Victoria de Durango, Durango, México. Número de teléfono: 6188121687, avitiaclaudia@gmail.com

Abstract

Protein tyrosine phosphatase 1B (PTP1B) is a negative regulator of insulin signaling. Thus, PTP1B is an attractive target to design new drugs against Type 2 diabetes. Our research group previously found a benzimidazole derivative molecule (JM151) that inhibits PTP1B. Due to its features such as Ki values in the low micromolar range (4.2 µmol) and drug like properties, this compound is a good candidate for optimization. However, there is a high structural homology between the catalytic domain of PTP1B with other phosphatases like T-cell-PTP (TCPTP), therefore, it was important to consider the interactions of some specific inhibitors reported for PTP1B to optimize JM151 molecule. To this end, molecular docking of JM151 was carried out in the two crystal structures of PTP1B (open and closed), using Glide software. The box included A, B, C y D sites in both conformations. A series of proposal derivatives of JM151 were analyzed. The compounds where the acid head in R_1 was oriented towards catalytic site (A site) were selected to evaluate their toxic potential by DataWarrior software. Subsequently, an induced fit docking procedure was applied. The compounds which interacted with Lys120, and those with a distance less than 6 Å from Phe52 were selected. Finally, these were undergone to a molecular docking analysis to choose molecules with different interactions in TCPTP active site. *In silico* analysis indicated that elongate the compounds structure and change the position of naphthyloxy groups from α to β and include a trifluoroethoxy-phenyloxy at position 4 in benzimidazole ring, improved their fitting into B site. Furthermore, removing the chlorine group at position 6 provided more torsional freedom and allowed binding near to Phe52 or Cys32 (also important for specificity). In conclusion, new potential and selective PTP1B inhibitors based on JM151 compound structure are reported.

Structural similarity search and biochemical characterization of methicillin resistant *Staphylococcus aureus* shikimate dehydrogenase inhibitors.

Daniel Isaac Enríquez Mendiola; Alfredo Téllez Valencia; Mónica Andrea Valdez Solana, Erick Sierra Campos, Claudia Isela Avitia Domínguez.

Facultad de Medicina y Nutrición, Universidad Juárez del Estado de Durango, Av. Universidad s/n, Centro, C.P. 34000, Victoria de Durango, Durango, México.
Tel: 6188121687; avitiaclaudia@gmail.com

Abstract

A highly pathogenic and clinically important microorganism is methicillin resistant *Staphylococcus aureus* (MRSA), responsible of causing different kinds of infections ranging from skin and soft tissues infections to other more invasive like septicemia, pneumonia and endocarditis. Due to its capability to create resistance to antibiotics, it is necessary to develop new drugs with specific targets in the metabolism of this microorganism. In this context, *S. aureus* depends on the shikimate pathway to synthesize aromatic compounds necessary to survive. Shikimate dehydrogenase (SDH), the fourth enzyme in this route, catalyzes the reversal reduction of 3-dehydroshikimate to shikimate, and is considered as an excellent target for the development of new antibacterial drugs. Therefore, the aim of the present work was to find through a similarity search strategy new inhibitors of MRSA shikimate dehydrogenase (SaSDH), taking into count the structure of three inhibitors previously reported by our group. Within the results, 50 molecules from the Chembridge library with more than a 50% of structural similarity were selected, and inhibition assays were carried out at a concentration of 100 µM. Two compounds (**31** and **37**) were capable of inhibiting the enzyme activity in a 98%. Through concentration curves, the IC_{50} (concentration that inhibits the 50% of the enzyme activity) was determined obtaining values of 25 µM and 21 µM for inhibitors **31** and **37**, respectively. Furthermore, a molecular docking protocol was carried out to determine enzyme-inhibitor interactions. The data showed that compounds made hydrogen bond interactions with important catalytic residues such as Lys 64 and Asp 100. Finally, their inhibition mechanisms were determined and biological activity assays were performed on MRSA cultures. In conclusion, the inhibitors reported serve as starting point to continue in the search of new drugs against MRSA.

Cooperativity in a dimeric enzyme is boosted by a novel mechanism

Jorge Marcos-Viquez[a], Annia Rodíguez-Hernández[b], Andrea Medina-García[a], Mario L. Calcagno[a], Adela Rodríguez-Romero[b] and Ismael Bustos-Jaimes[a,1]

a Department of Biochemistry, School of Medicine, UNAM
b Department of Biomacromolecules, Institute of Chemistry, UNAM
1 70-159, Mexico City P.C. 04510; Tel/phone: (55)56232260; Email: ismaelb@unam.mx

Abstract

The GlcN6P deaminase (EC 3.5.99.6, NagBI) catalyzes the isomerization-deamination of glucosamine 6-phosphate (GlcN6P) releasing fructose 6-phosphate (Fru6P) and ammonium (NH_3^+). In *E. coli*, NagBI is a homohexamer with Rossmman-like fold and constitutes a regulatory metabolic step because of both its positive cooperativity and its allosteric K activation by *N*-Acetylglucosamine-6-phosphate (GlcNAc6P). In contrast, the same activity has been found in dimeric proteins with Sugar Isomerase (SIS) fold in bacteria of the *Shewanella* genus (NagBII). These enzymes are also activated by GlcNAc6P and display homotropic cooperativity by its substrate GlcN6P, with a Hill Coefficient ≥ 2, higher than the maximum number of sites for a dimer. NagBII enzymes constitute special examples of non-homologous isofunctional enzymes, as the convergence is not only in catalysis but also in their cooperativity and allosteric properties. We studied the molecular mechanisms of activation and cooperativity by enzyme kinetics, isothermal titration calorimetry, and X-ray crystallography. ITC experiments revealed the existence of a single binding site per monomer for the allosteric activator GlcNAc6P. In contrast, there are two binding sites per monomer for the competitive inhibitor 2-amino-2-deoxy-glucitol-6-phosphate (GlcNol6P) with distinctive thermodynamic signatures, indicating the existence of two clearly different sites. Crystallographic structure of the ligand-free enzyme as well as the enzyme complexed with both the activator and the competitive inhibitor, clearly demonstrated the identities of the active and allosteric sites. Moreover, the structure of the enzyme complexed only with GlcNol6P revealed that this molecule, that resembles the substrate and binds to the active site, also binds to the allosteric site. This information was used to propose a kinetic model that includes all the occupation states of the enzyme and explains its kinetic behavior. This is a new mechanism to generate a high degree of cooperativity in an enzyme with a reduced number of active sites.

Precise design of protein and DNA nanomaterials through the engineering of protein polymers and DNA binding domains

Armando Hernández-García[1,2] & Renko de Vries[2]

1) Wageningen University, Laboratory of Physical Chemistry and Soft Matter, Stippeneng 4, 6708 WE, Wageningen, the Netherlands.
2) Institute of Chemistry, Laboratory of Biomolecular Engineering and Bionanotechnology, UNAM, Circuito Exterior, Ciudad Universitaria, Delegación Coyoacán C.P. 04510. Tel. +52 55 56224548, e-mail: armandohg@iquimica.unam.mx

Emerging DNA-based nanotechnologies would benefit from the ability to modulate the properties (e.g., solubility, melting temperature, chemical stability) of DNA templates (single molecules or origami nanostructures) through controlled, self-assembling coatings. We here introduce a DNA coating agent, called C8–BSso7d, which binds to and coats with high specificity and affinity, individual DNA molecules as well as folded origami nanostructures. C8–BSso7d coats and protects without condensing, collapsing or destroying the spatial structure of the underlying DNA template. C8–BSso7d combines the specific non-electrostatic DNA binding affinity of an archeal-derived DNA binding domain (Sso7d, 7 kDa) with a long hydrophilic random coil polypeptide (C8, 73 kDa), which provides colloidal stability (solubility) through formation of polymer brushes around the DNA templates. C8–BSso7d is produced recombinantly in yeast and has a precise (but engineerable) amino acid sequence of precise length. Using electrophoresis, AFM, and fluorescence microscopy we demonstrate protein coat formation with stiffening of one-dimensional templates (linear dsDNA, supercoiled dsDNA and circular ssDNA), as well as coat formation without any structural distortion or disruption of two-dimensional DNA origami template. Combining the programmability of DNA with the nonperturbing precise coating capability of the engineered protein C8–BSso7d holds promise for future applications such as the creation of DNA–protein hybrid networks, or the efficient transfection of individual DNA nanostructures into cells.

Therapeutic model anti tumoral targeting E5 protein of human papillomavirus 16 to dendritic cells *in vivo*

Oscar Badillo-Godínez[1], Lilia Chihu-Amparán[1], Adolfo Pedroza-Saavedra[1], Laura Bonifaz-Alfonzo[3], Fernando Esquivel-Guadarrama[2] Lourdes Gutiérrez-Xicotencatl[1].
[1].- CISEI-INSP, SSA, Cuernavaca, Morelos [2].- Facultad de Medicina, UAEM, Cuernavaca, Morelos. 3.- Unidad de Investigación en Enfermedades Autoinmunes, Hospital de Especialidades, Centro Médico Nacional siglo XXI, IMSS, México.
badillo.oscar@gmail.com. Centro de Investigación Sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Av. Universidad 655, Col. Sta. María Ahuacatitlán. Cuernavaca, Morelos, México. C.P. 62100. Phone: (777) 329-30-00 Ext. 2263.

Key Words: Targeting, DEC-205, Dendritic Cells, E5 HPV-16, Tumor.

The uterine cervical cancer is one of the most important health problems worldwide. The human papillomavirus (HPV) is the main etiological agent in the development of cervical intraepithelial neoplasia (CIN) and cervical cancer (CC). The HPV codify for three oncogenes E5, E6, and E7, which are specific tumor targets for vaccine design. The E6 and E7 are expressed all along the different stages of the disease, while, E5 is expressed on premalignant lesions (CIN I-II) and condylomas. The E5 oncoprotein from HPV type 16 is a hydrophobic membrane protein of 83 amino acid, located at the endoplasmic reticulum, Golgi, endosomal compartment and plasma membrane. E5 interferes with the activation of T cells by downregulating the MHC-I and II, which allows the establishment of a persistent infection, later on, the cellular transformation and finally the development of cancer. Recently, a therapeutic mouse model against the DEC-205 receptor has been described and targets antigens to dendritic cells (DCs). This system induces a potent T cell activation and a protective immune response at the systemic level, even when inoculated parenterally. The aim of the present work was to evaluate whether E5 could be targeted to DEC-205, present in the DCs, and induce a protective immune response against an HPV tumor in a BALB/c mouse model, generated by transference of BMK16-myc cells that contain the complete HPV16 genome. To generate the tumors, groups of 5 BALB/c mice were inoculated with 5x105 BMK16-myc cells s.c., and 7 days later immunized with different treatments as follows: 1) Targeting 5 µg of anti-DEC-205-E5; 2) Five µg of E5 alone; 3) Five µg antibody isotype-E5; 4) Targeting anti-DEC-205-/E5+ VP6 as immune-modulator and 5) PBS. All the treatments were administrated with 50 µg of Poly I:C as adjuvant. Mice were re-immunized one week later with the same treatment and follow up for up to 100 days by measuring the tumor size weekly. A group of mice was sacrificed at day 30 to evaluate the T cell activation and production of cytokines *in vitro* by anti-E5 T cells from peripheral lymph nodes. The mice immunized with DEC-205-E5, or DEC-205-VP6/E5 showed a reduction of tumor size between 10 to 15 times, respectively and there was an increase in T cells activation, and production of cytokines IL-17 and IFNγ as compared with the E5, isotype-E5 and PBS treated mice. In the DEC-205-E5 and DEC-205-VP6/E5 groups, the 66% and 80% of mice, showed almost a complete clearance of the tumor and this was observed up to 72 days after immunization, respectively. In contrast, the tumor-bearing mice immunized with E5 alone, isotype-E5 or PBS showed no reduction in tumor growth. These results showed that E5 targeted to DCs through the DEC-205 receptor could induce a strong protection against E5 and increase this effect when co-inoculated with VP6 as an immune-modulator and lead to a significant inhibition of tumor growth and increased survival (100 vs 30 days) in this murine tumor model. These results could contribute in the future for the design of a new generation of HPV therapeutic vaccine.

Apoptosis of mouse hippocampal cells induced by Taenia crassiceps metacestode factor

Natalia Ivanovna Copitin Niconova, Nadia Zepeda Córdova, Sandra Georgina Solano Gálvez, José Luis Chávez Juárez, Ana María Fernández Presas, Fernando García Hernández, Patricia Tato Zaldívar, José Luis Molinari Soriano

Departamento de Bioquímica y Biología Estructural, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México. Ciudad de México.
Circuito Exterior s/n Ciudad Universitaria, Coyoacán, 04510 México D.F. Teléfono: 56225653. Correo electrónico: jmolinar@ifc.unam.mx

Seizures, headache, depression and neurological deficits are the signs and symptoms most frequently reported in human neurocysticercosis. However, the cause of the associated learning and memory deficits is unknown. Here, we used *Taenia crassiceps* infection in mice as a model of human cysticercosis. The effects of T. *crassiceps* metacestode infection or T. *crassiceps* metacestode factor (MF) treatment on mouse hippocampal cells were studied; control mice were included. At 45 days after infection or treatment of the mice with MF, all mice were anaesthetized and perfused transcardially with saline followed by phosphatebuffered 10% formalin.

Then the brains were carefully removed. Coronal sections stained using several techniques were analysed. Extensive and significant apoptosis was found in the experimental animals, mainly in the dentate gyrus, CA1, CA2, CA3 and neighbouring regions, in comparison with the apparently intact cells from control mice ($P < 0.01$). These results suggest that neurological deficits, especially the learning and memory deficits, may be generated by extensive apoptosis of hippocampal cells.

Searching for the natural ligand of the Human Aminopeptidase N (CD13)

López- Cortés, Georgina Ivette. Ortega- Soto, Enrique.
Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México,
Circuito escolar, Ciudad universitaria, Ciudad de México, México. 5622- 3834 ortsoto@unam.mx

Introduction The Aminopeptidase N (CD13, gp 150, ANPEP) is a membrane protein expressed on different cell types in humans including intestinal and renal epithelial cells, neurons of the CNS and on myelomonocytic cells. CD13 is a highly glycosylated protein characterized as an enzyme that cleaves N-terminal neutral amino acids from peptides and proteins. CD13 acts also as a viral receptor and it is involved in angiogenesis, tumor cell invasion, cell adhesion and migration (Mina- Osorio, P., 2008), it has been shown to mediate signal transduction as well as causing cytoskeleton's rearrangements. (Navarrete-Santos, A., *et al*. 2000, Licona-Limón, I., *et al.* 2015). However, the evidence for CD13 effector functions such as has been obtained either by monoclonal antibodies (MoAb) or by genetically modified animals or with enzymatic inhibitors, since the natural ligands that could induce those functions have not been identified.
Purpose The aim of this study is to identify amino acid sequences that could be part of the natural ligand of human CD13.
Methods The strategy of selecting peptide sequences was accomplished by Phage Display technology over transfected cells (HEK 293) with the CD13 gene. We used two different libraries: a 12 amino acid sequence and another made up with human brain cDNA. Next, some selected clones were sequenced and analyzed by alignments.
In order to test whether the physiological substrates of CD13 cause or do not cause a signal transduction cascade, the activation of Src was quantified in cells incubated with three different substrates and an inhibitor of the enzymatic activity. By other means, the localization of membrane CD13 was visualized on these cells after incubation with the same substrates and inhibitor.
Results After three rounds of selection, clones were selected to prove an specific affinity to CD13. Individual clones were tested for their specific union to CD13+ cells and not to CD13- cells. Consequently, the clones with a higher specific union to CD13+ cells were sequenced and aligned in order to know the peptides with the higher identity values. We came up with 6 different peptides that were studied so as to know if the interaction with CD13 could cause the effector functions reported. From those 6 peptides, two were discarded because they resemble a non coding sequence of the mRNA of the peptide and the others sequences are still in consideration.
To evaluate if the substrates could elicitate a signal cascade, the quantification of activated Src of cells incubated with the substrates was measured by flow cytometry and the localization of membrane CD13 was visualized by immunofluorescence microscopy. Just as the negative control, the distribution of CD13 in the membrane in cells incubated whether with the substrates or with the inhibitor is homogenous with few points of CD13 aggregation. In the contrary, aggregation of CD13 in the membrane caused by Ab 452 is evident.

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Lipids induce the formation of germinal centers in the same time as a protein antigen

Claudia Albany Reséndiz Mora, Viridiana Galicia Galicia, Sandra Laura Vela Patiño, Monserrat Figueroa Pensado, Luis Lamas Garcia, Isabel Baeza & Carlos Wong Baeza.

Laboratorio de Biomembranas, Departamento de Bioquímica, Escuela Nacional de Ciencias Biológicas – Instituto Politécnico Nacional.
Unidad Profesional Lázaro Cárdenas, Prolongación de Carpio y Plan de Ayala s/n, Col. Santo Tomas C.P. 11340 México City. México. charlywong@icloud.com

In some infectious or autoimmune diseases, IgG anti-lipid antibodies have been found that can participate in the physiopathology, especially of the latter. In our research group, a mouse model was developed that shows a pathology similar to systemic lupus erythematosus; this was achieved by the administration of liposomes with non-bilayer phospholipid arrangements called "lipid particles" induced and stabilized with the drug chlorpromazine (CPZ); One of the characteristics of the mice of this model is the presence of anti-lipid particle IgG class antibodies formed mainly via germinal center. Since the immunogenicity mechanisms of lipid antigens such as lipid particles are poorly understood, their study could contribute not only to the understanding, but also to the diagnosis and treatment of some pathologies. Therefore, our objective in this work is to identify cells from germinal centers that respond at different times against lipids associated with lipid particles. For this, BALB / c mice were administered with the lipid antigen (liposomes with chlorpromazine-stabilized-lipid particles) (Group CPZ), with the ovalbumin protein antigen (Group OVA, as a positive control of the germinal center response) and as negative controls, one group was administered with saline-solution (Group SS) and the last one did not receive administrations. The spleen and mesenteric lymph nodes were extracted to perform cytometry and hematoxylin and eosin staining at different post-administration times. In the negative controls, no change in the size of the lymphoid organs was observed. In the mice administered with liposomes with lipid particles, the lymphoid organs increased in size and showed an increase in the germinal center cells at 15 days post-administration, whereas in the mice administered with OVA the cellular increase was observed at a shorter time (10 days). The extrafollicular response induced by both antigens (lipid and protein) is similar in the different post-administration times. Histologically, germinal centers were found in the CPZ and OVA groups, starting 7 and 5 days after administration. As conclusions we found a difference in the percentages of cells of interest for each study group, these were greater for the CPZ and OVA groups. The variation of the percentage of cells studied in the different groups (SS, CPZ, and OVA) indicates that an immunological process is carried out, however with the results obtained, it is not possible to determine if there is activation or migration of the cells studied, so it is necessary to carry out other types of studies, as well as complement those already obtained.

A *Taenia crassiceps* factor induces apoptosis of spleen CD4+ T cells and TGF-β and Foxp3 gene expression in mice

Nadia Zepeda Córdova, Rocío Gabriela Tirado Mendoza, Natalia Ivanovna Copitin Niconova, Sandra Georgina Solano Gálvez, Ana María Fernández Presas, Patricia Tato Zaldívar, José Luis Molinari Soriano.
Instituto de Fisiología Celular, Universidad Nacional Autónoma de México.
Circuito Exterior s/n Ciudad Universitaria, Coyoacán, 04510 México D.F. Teléfono: 56225653.
Correo electrónico: jmolinar@ifc.unam.mx

The study was undertaken to determine whether a parasite substance produces structural pathology in the mouse spleen. A low-molecular-weight *Taenia crassiceps* metacestode factor (MF) isolated from the peritoneal fluid of female mice infected with *T. crassiceps* metacestodes induced pathological and immunological changes in mouse spleen cells in vivo. Electron microscopy and confocal microscopy revealed severe changes in the spleen histoarchitecture of *T. crassiceps*-infected and MF-treated mice. Apoptotic degenerated spleen cells were observed in the white and red pulps and were more conspicuous in the white pulp of the spleen from the *T. crassiceps*-infected mice than in that of the MF-treated mice. Flow cytometry analysis revealed that the numbers of spleen CD4+ Tcells were significantly lower in both experimental groups than in control mice. The ex vivo expression of transforming growth factor β (TGF-β) and factor Foxp3 were significantly higher in splenocytes of the experimental mice than the basal expression observed in the control cells. These findings may have potential applications for a better understanding of the host–parasite relationship in human neurocysticercosis.

In vitro development of splice-switching oligonucleotides to revert intronic DRD2 polymorphisms effect associated to Substance Use Disorders

Alejandro Bustos Cortés, Selma Eréndira Avendaño Vázquez.
Instituto Nacional de Medicina Genómica (INMEGEN).
Periférico Sur 4809, Arenal Tepepan, 14610 Ciudad de México. Tel. 01 555350 1900, ext. 1900.
[seavendano@inmegen.gob.mx].

Keywords: *Alternative Splicing, DRD2, splice-switching oligonucleotides.*

Background. Up to 50% human genetic diseases are caused by mutations that affect splicing (*Disterer P, 2014*). Most pre-mRNAs can be spliced in different ways to produce distinct mature mRNA isoforms in a process called alternative splicing. Mutations that occur within introns are usually assumed to alter splicing patterns, that have been estimated to account for up to a third of all disease-causing mutations (*Havens MA, 2013*). The intronic variants contribution to disease is generally unknown for non-Mendelian disorders, such as Substance Use Disorders (SUD) (*Moyer RA, 2011*). DRD2 has been linked to SUD and two isoforms of DRD2 have been studied: short (D2S) and long (D2L) isoforms, that arise from the alternative splicing of exon 6. In humans, two intronic polymorphisms that influence D2S/D2L splicing have been discovered: rs1076560 and rs2283265. Genetic and genomic approaches support the hypothesis that these polymorphisms reduce D2 expression and increase SUD risk (*Clarke TK, 2014*). SSOs are short antisense oligonucleotides designed to create a steric block to the binding of splicing factors to the pre-mRNA (*Havens MA, 2016*). Accordingly, we hypothesized that intronic polymorphisms which alter D2S/D2L splicing pattern creates de novo regulatory cis elements that can be modify using SSOs.

Aim. Manipulate the D2L/D2S splicing profile using splice-switching oligonucleotides.

Methods. Cell culture. Human neuroblastoma SH-SY5Y cells were cultured in DMEM-HG/F12K media containing 10% FBS or serum replacement and 1% penicillin-streptomycin under 5% CO2. **DRD2 minigene splicing.** We use QuikChange, a ligase independent cloning protocol, to introduce exons 5-7 and introns 5 and 6 in pEGFP-N1, and then to introduce rs1076560 and rs2283265. **SSOs design.** We design oligonucleotides with 2'-MOE-PS modifications to block target sites of neuronal splicing regulators presents between intron sequences as well as both polymorphisms. **Detection of splice isoforms.** D2S and D2L were detected by PCR amplification of cDNA with specific primers.

Results. We obtain a partial gene construct containing exon 5 to 7 and carrying or not the polymorphisms rs1076560 and rs2283265 in an expression vector. These constructs were transient transfected into SH-SY5Y cells. We extracted RNA from the cells, made cDNA using anchored oligo(dT)20 primer and detection of D2S/D2L isoforms using end-point PCR. We could not identify D2S/D2L endogenous expression and minigenes were not processed efficiently in SH-SY5Y. We hypothesized that any component in FBS inhibits DRD2 expression modulating splicing regulators relevant to D2S/D2L expression. Therefore SH-SY5Y cells were cultured in medium containing 10% FBS or serum replacement. Interestingly cells in serum replacement shown changes in morphological traits and the relative expression of D2 isoforms increase in serum replacement compared to FBS.

Perspectives. We will perform a transient transfection into SH-SY5Y cells cultured in medium supplemented with serum replacement to evaluate SSOs on D2 expression.

Finding a 5-hydroxymethylcytosine "fingerprint" during hepatocyte differentiation. A clue for early cancer detection and intervention?

Jesús Rafael Rodríguez-Aguilera[1,2], Szilvia Ecsedi2, Marie-Pierre Cros[2], Chloe Goldsmith[3], Nuria Guerrero-Celis[1], Rebeca Pérez Cabeza-de-Vaca[1], Félix Racillas-Targa[1], Héctor Hernández-Vargas[3]*, and Victoria Chagoya de Sánchez[1#].
[1]Instituto de Fisiología Celular, Universidad Nacional Autónoma de México. [2]International Agency for Research on Cancer, Epigenetics Group. [3]Centre Léon Bérard, Department of Translational Research and Innovation. [1]Circuito Exterior s/n Ciudad Universitaria, Coyoacán, 04510 Ciudad de México. *hector.hernandez-vargas@lyon.unicancer.fr, #+525556225614, vchagoya@email.ifc.unam.mx

Purpose: Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the second in mortality, due in part to late diagnosis and lack of a curative therapy. Recently it has been described that DNA modifications, and specifically 5-hydroxymethylcytosine (5hmC), are required for the establishment and maintenance of parenchymal liver cell identity through modulation of expression of the master regulator of hepatocyte differentiation *HNF4A*. We hypothesize that loss of hepatocyte identity is an early event in HCC development; therefore, 5hmC may represent a useful biomarker. To that end, we aimed to identify dynamic 5hmC loci associated with hepatocyte differentiation. In parallel, we analysed the effects on this modification by an anti-fibrotic/anti-carcinogenic adenosine derivative, IFC-305. As this compound is able to modify DNA methylation triggering liver disease reversion, we asked whether it also influences hepatocyte identity.

Methods: We differentiated the liver progenitor cell line HepaRG in absence or presence of IFC-305. We carried out DNA isolation and performed conversion with oxidative bisulfite, to distinguish 5mC, 5hmC and non-modified cytosines. We further processed all samples for methylome-wide analysis.

Results: We obtained 3351 differentially methylated positions (DMP) and 818 differentially hydroxymethylated positions (DhMP) between proliferative and differentiated cells, whereas the comparison between IFC-305 treated and non-treated cells revealed 1348 DMPs and 548 DhMPs. Finally, when we compared proliferative vs. IFC-305 treated cells, we identified 2290 DMPs and 609 DhMPs.

Conclusions: Our data indicates that there is demethylation and enrichment of hidroxymethylation during HepaRG differentiation, whereas IFC-305 treatment generates an intermediate state between proliferative and differentiated cells. This effect suggest a capacity of IFC-305 to regulate cell identity, which could be involved in its hepatoprotective properties.

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Participation of the epigenetic factor BORIS in transcriptional regulation of genes involved in ovarian cancer.

Ernesto Soto-Reyes*[1,2], Marisol Salgado-Albarrán*[1,2], Rodrigo González-Barrios[2], Nicolás Alcaráz[3], Lissania Guerra-Calderas[1,2], Clementina Castro[2,4], Elena Arechaga-Ocampo[1], Yesennia Sánchez-Pérez[2],Alejandro García-Carrancá[2,4], Jan Baumbach[5]. *Co-first authors.

[1].Natural Sciences Department, Universidad Autónoma Metropolitana (UAM), Mexico City 05300, Mexico. [2].Cancer Biomedical Research Unit, Instituto Nacional de Cancerología (INCan), Mexico City, Mexico. [3].The Bioinformatics Centre, University of Copenhagen, Copenhagen, Denmark. [4].Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México (UNAM), Mexico City, Mexico. [5].Chair of Experimental Bioinformatics, TUM School of Life Sciences, Technical University of Munich, Munich, Germany.
Corresponding author: Soto-Reyes, E. UAM-C, Av. Vasco de Quiroga 4871, Col. Santa Fe, Del. Cuajimalpa de Morelos, 05348, CDMX. email. esotoreyes@correo.cua.uam.mx

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Cancer exhibits a deregulation in genetic and epigenetic components that lead to an uncontrolled cell proliferation. CTCF is an epigenetic component that is deregulated in multiple neoplasms. In the cells, CTCF can establish chromosomal territories through the interaction of its 11 zinc fingers and the recruitment of partner proteins. *CTCF* has a paralog gene known as *CTCF-Like* or *CTCFL*, which codes for the BORIS protein. BORIS is a transcriptional regulator with low or null expression levels in somatic cells; however, it has been shown that several types of cancers, such as ovarian cancer, which exhibit an increase on its expression. This overexpression is related with poor prognosis, suggesting that BORIS could have an important role in transcriptional regulation of ovarian neoplastic cells. However, the gene targets that could be altered by BORIS are unknown. Thus, the aim of this project was to identify the genes regulated by BORIS in an ovarian cancer cell line and ovarian cancer patients. To address this question, we decreased the expression of BORIS by siRNA (Knock Down, KD) or CRISPR / Cas9 system (Knock Out, KO). By microarray expression analysis we identified 299 genes that were differentially expressed in KD cells (192 positively regulated and 107 negatively regulated) and 418 genes (262 positively regulated and 156 negatively regulated) in KO cells. From these genes, 130 genes were shared in both cellular models. Using a bioinformatic analysis (*de novo Pathway Enrichment Analysis)* we obtained a BORIS-associated regulatory network; which showed that the Androgen Receptor (AR) acts as a main transcription factor. Also, *FN1*, *FAM129A* and *CD97* genes were identified as relevant genes in the network. These genes have been reported altered in ovarian cancer and could be used as potential biomarkers. Therefore, to determine if *CTCFL*, *AR*, *FN1*, *FAM129A* and *CD97* genes could be used as prognostic biomarkers we evaluated their expression profiles in ovarian cancer samples by qRT-PCR. We found that the expression of these genes is closely related to the expression of *CTCFL*. This suggests that BORIS could act as a master regulator of genes involved in ovarian cancer. As a conclusion, our data proposes BORIS as a relevant transcriptional regulator in ovarian cancer patients, mainly by the repression of Androgen Receptor and other genes such as *FN1*, *FAM129A* and *CD97*.

Epigenetic modulation of Ca2+ homeostasis genes by Resveratrol helps to explain its anticancer properties

Ángel Zarain-Herzberg*1, Eduardo Izquierdo-Torres1, Gabriela Rodríguez Rodríguez1, Ana Catalina Arreola Tapia1, and Iván Meneses Morales1.
1Department of Biochemistry, School of Medicine, National Autonomous University of Mexico. Mexico City, 04510, México. Tel.: +52 (55) 5623-2258, Fax: +52 (55) 5616-2419, E-mail: zarain@unam.mx

Resveratrol (RSV) is a phytoestrogen which is able to modulate cellular processes involved in all stages of carcinogenesis [1]. Previously, we demonstrated that RSV upregulates specifically the expression of *ATP2A3* gene in MCF-7 and MDA-MB-231 breast cancer cells. This up-regulation is important for RSV-induced apoptosis since silencing of SERCA2 and SERCA3 Ca^{2+} pumps using siRNAs partially decreased the apoptotic process [2]. Evidence suggesting that the modulation of gene expression induced by phytoestrogens involves epigenetic modifications is widely available [3, 4]. In this work, we explore the molecular mechanism for RSV-induced *ATP2A3* up-regulation in MCF-7 and MDA-MB-231 breast cancer cell lines.
Our results indicate that HDAC activity in nuclear extracts of these breast cancer cells treated with RSV was reduced, as well as HAT activity was increased. The reduced HDAC activity could be attributed to the reduction of HDAC2 protein in nuclei of cells treated with RSV, compared to untreated cells, as well as changes in HDAC2 localization since IF experiments showed a cytosolic accumulation of HDAC2 in MCF-7 cells treated with TSA or RSV . The global acetylation pattern on histone H3 is increased in both cell lines treated with RSV or TSA compared with untreated cells. When we evaluated, through ChIP assay, the enrichment of histone marks for transcriptional activation (H3K9Ac and H3K27Ac) in the proximal promoter (region encompassing from -258 to -143) of *ATP2A3* gene in MCF-7 and MDA-MB-231 cells, we found an increase in H3K27Ac in MDA-MB-231 cells treated with RSV or TSA, but not in MCF-7 cells. H3K9Ac remained unchanged in both cell lines. HDAC2 relative occupancy decreased in the same region in MDA-MB-231 cells.
The results of this work help to explain the benefit of phytoestrogens consumption observed in epidemiological data and studies with animals and *in vitro* models.

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Long term effect of early overnutrition in the transcriptome of Wistar rat liver (Rattus norvegicus).

[1,4]MSc. Miguel Angel Espinoza Camacho, [3]MSc. Gerardo Sanchez Garcia, [2]PhD. Luis Antonio Reyes Castro, [2] PhD. Elena Zambrano González, [1]PhD. Maria Elizabeth Tejero Barrera, [3]PhD. Enrique Hong Chong and [1]PhD. Laura del Bosque Plata.

[1]*National Institute of Genomic Medicine. Mexico.*
[2]*National Institute of Medical Sciences and Nutrition "Salvador Zubirán". Mexico.*
[3]*Center of Research and Advanced Studies. Mexico.*
[4]*National Autonomous University of México. Mexico.*
Email: ldelbosque@inmegen.gob.mx, mail: Periferico Sur 4809, Arenal Tepepan, Tlalpan, 14610 Mexico City. Mexico.

Key words: overnutrition, transcriptome, liver.

Background

Obesity disease is a global health problem that leads to the generation of diabetes and hypertension. The overnutrition is an environmental that is characterized by a consumption that exceeds the energy requirement and contributes to the generation of obesity.
The short term effect overnutrition influences the release of glucose and lipoproteins in the liver. It regulates thermogenesis in brown adipose tissue and lipolysis in white adipose tissue, promote oxidation of lipids in the skeletal muscle and regulates food intake.
Studies in animals exposed to early overnutrition show evidence of the development of hyperphagia, overweight and inhibition of satiety manifested by high levels of leptin.
The main objective of this work was to analyze the long-term effect of the early postnatal overnutrition (EPO) in the liver tissue transcriptome.

Materials and methods

EPO model in Wistar rats by overlactation from day 3 to day 21 of life by litter size reduction method using pup male was generated; control (n = 4) and overnutrition (n = 6). After 21 days the rats had a diet and water ad libitum until 8 months. The rats were euthanized using the decapitation technique. Transcritome was analyzed by microarrays hybridization to explore gene expression with Ratgene ST 1.0, Affymetrix® chip. The pathway enrichment analysis was performed with the Ingenuity Pathway Analysis software (IPA) and DAVID bioinformatic data base. Contrast bioinformatic analysis was performed with R statistical software, statistical analysis for phenotypic and biochemical parameters were analyzed in Graphpad Prism 6® software was applied Student t probe with $p < 0.05$.

Conclusions

EPO increased the concentration of triglycerides and serum insulin in the treatment group.
EPO induces 53 genes differentially expressed in the hepatic transcriptome, 23 genes were over expressed and 30 genes were sub expressed. (using cutoff of log 2 fold change of <0.3> and p= <.05.
The analysis of the interaction networks in the liver transcriptome had 8 mainly abnormal networks related to lipid metabolism and chronic inflammation in the treatment group.
Particularly the pathway of triglyceride biosynthesis has positive enrichment, which explains the high concentration of triglycerides in the treatment rats.
EPO generates a metabolic programming in the lactation stage that in the long term in the adult life of rats stimulates the production of triglycerides in the liver and generates a subsequent systemic insulin resistance.

Comparison of microRNAs quantity in blood serum on patients from 65 years and older with frailty and patients clinically functional.

Authors: García-Gamboa Ada Paulina, Rivera Sánchez José de Jesús, Fonseca-Sánchez Miguel Ángel, Queipo García Gloria Eugenia *.

Hospital General de México
Dr. Balmis #148 Col. Doctores, Del. Cuauhtémoc, CDMX.C.P.06720.Tel:2789 - 2000 Ext. 5802 e-mail: gqueipo99@icloud.com, a.paulinag@yahoo.com

Introduction: Around the globe the population tendencies are changing, with an increase in the group of 60 years and older. Thus, investigations are focusing efforts in making a better quality of life for this group. Aging comes with several modifications on the physiology of the body, some of which lead to deficiencies and particular illnesses. One of the most relevant is frailty and muscle loss. In Mexico the National Institute for Statistics and Geography, INEGI estimates the percentage of people with frailty is near 12.5% in the ages between 65 and 69 years and 47.8% older. Frailty is characterized by a progressive multi-systemic deterioration, with decline of the physiology reserves and poor resiliency, along with muscle loss in most of the cases. Recently there was an evaluation of miRNAs in this group of patients with muscle biopsy where there was found a difference in the quantity of some miRNAs between healthy patients and those with frailty. However muscle biopsy is too invasive for its use for every patient.

Objective: We aim to obtain miRNAs from serum from serum of elder healthy patients and those with frailty and analyze them by RT-PCR to evaluate its presence and compare the quantity on each group for future use in the detection and prognosis for frailty and muscle loss using less invasive methods.

Methods: This study is analytic, transversal and observational. Sample size was 130 subjects from 65 years and older, which was divided in two groups, the healthy and the frail. Patients were recruited from the medical assistance on the Geriatrics Department at the General Hospital. They were classified and invited through informed consent, and given time to decide voluntarily to participate. After the signing of the consent blood sample was obtained by venous puncture and then it was centrifuged to get the serum. MicroRNA's were obtained by the TRIzol LS method and quantified by qRT-PCR was performed in every sample. Afterwards the quantities from each sample will be analyzed by Double Delta CT to detect discrepancies of the groups.

Results: We analyze 130 samples through qRT-PCR, expression was analyzed subsequently by the Double Delta CT. Discrepancies were analyzed statistically using ANCOVA to eliminate miscalculations.

Maize root exudates. Large-scale phenotyping of a tropical and temperate diversity panels.

Martha G. Lopez-Guerrero[1], a, Peng Wang[1],b, Sophie Alvarezb, Daniel P. Schachtmanc, Karin van Dijka
[1]co-contributors.
[a]Department of Biochemistry, University of Nebraska-Lincoln. bDepartment of Agronomy and Horticulture, University of Nebraska-Lincoln. cDepartment of Agronomy and Horticulture and Center for Plant Science Innovation, University of Nebraska-Lincoln.
Martha G. Lopez-Guerrero. 1901 Vine Street. N161. Beadle Center. University of Nebraska-Lincoln, NE 68588-0664. martha.lopez-guerrero@unl.edu, marsg.loguerrero@gmail.com. +1(402) 499 5598, +52 (777) 135 8030.
PI. Karin van Dijk. 1901 Vine Street. N147 Beadle Center. University of Nebraska-Lincoln, NE 68588-0664. kvandijk2@unl.edu. +1 (402) 472-2948.
PI. Daniel P Schachtman. 1901 Vine Street. Beadle E243. University of Nebraska-Lincoln, NE. 68583-0660. daniel.schachtman@unl.edu. +1 (402) 472 1448.

Root exudates may play important roles in nutrient acquisition, stress tolerance and in the interaction with microorganisms. However, the root exudate chemical diversity within important crop species such as Zea mays has not been thoroughly investigated. Our aim is to characterize the root exudate composition of 150 tropical genotypes and about 150 temperate lines from the Buckler-Goodman diversity panel. We designed a semi-hydroponic plant growth system for exudate collection. The plants were grown in a glass beads matrix for two weeks with a semi-automatic watering system. Using targeted analysis, GC-MS and LC-MS, we have detected 9 hormones (including Indole-3-Acetic Acid, Jasmonic Acid and, Salicylic Acid), 19 amino acids (including Proline), 3 organic acids (Fumaric acid, Succinic acid, Malic acid) and, 10 sugars (including Sucrose and Fructose). The results suggest that there are differences in the abundance and composition for exudates between lines. Results will also be presented on any correlations detected between root architecture and exudate profiles.

Low levels of polyamine alter resistance in Arabidopsis to necrotrophic and hemi-biotrophic pathogens

Ana Isabel Chávez Martínez[1], María Azucena Ortega Amaro[1], Alicia Becerra Flora[1], Martha Torres Basaldúa[2], Mario Alberto Serrano Ortega[2] and Juan Francisco Jiménez Bremont[1]

[1] División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica (IPICYT) AC, Camino a la Presa de San José 2055, C.P. 78216. San Luis Potosí, SLP, México. (+52) 444-8-342000. jbremont@ipicyt.edu.mx
[2] Center for Genomic Sciences, The National Autonomous University of Mexico UNAM, Campus Morelos. (777) 3115164. ZIP 62210. Cuernavaca, Morelos, México.

Plants are sessile organisms that have developed strategies to resist the attack against pathogens. Changes in polyamine levels have been reported during the interaction of plant with microorganisms. Polyamines (PAs) are low weight polycations that participate in plant defense against pathogens. The most abundant PAs are putrescine (Put), spermidine (Spd) spermine (Spm) and thermospermine (tSpm). We generated a non-lethal line (amiR:*ADC*-L2) of Arabidopsis that has a drastic reduction in putrescine, spermidine and spermine content. Recently, we have found that amiR:*ADC*-L2 silenced line shows increases in H_2O_2 and O_2^- content. In this work, the susceptibility of the amiR:*ADC*-L2 line to *Pseudomonas syringae* and *Botrytis cinerea* was investigated. The amiR:*ADC*-L2 line showed an incremented susceptibility to *Botrytis cinerea* compared to the parental wt plant. In contrast, an increased resistance of the silencing line to infection by *Pseudomonas syringae* was observed with respect to wt control plants. Subsequently, both phenotypes were reversed by the Put pre-treatment. Afterwards, the expression of molecular markers involved in the pathways of jasmonic acid, ethylene and salicylic acid in amiR:*ADC*-L2 line after *B. cinerea* and *P. syringae* infection was analyzed. These data suggest that the decrease of PAs levels in amiR:*ADC*-L2 line alters the response to the interaction with *P. syringae* and *B. cinerea*.

Crosstalk between Methyl jasmonate and Polyamines on the Arabidopsis growth

Maria Azucena Ortega Amaro, Silvia Pérez Sánchez, Ana Isabel Chavez Martinez, Montserrat Chacón Flores, Alicia Becerra Flora and Juan Francisco Jiménez Bremont.
División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica (IPICYT) AC, Camino a la Presa de San José 2055, C.P. 78216. San Luis Potosí, SLP, México. (+52) 444-8-342000. jbremont@ipicyt.edu.mx

Jasmonates (JAs) such jasmonic acid (JA) and its methyl esther are known to participate in plant defense mechanisms, and have recently been reported to inhibit plant growth as well as seed germination at non-physiological concentrations. Like other phytohormone, they do not participate alone in the activation of the processes that they regulate, but they can also interact with other signaling molecules. In several studies the interaction of JAs with other phytohormones such as ethylene, salicylic acid, auxins or abscisic acid has been studied, however the molecular mechanisms of JAs with the other hormones are currently unknown. The exogenous application of polyamines (PAs) has given positive results in growth, development and protection against diverse biotic and abiotic stress in multiple crops. In this work, the effect of the addition of PAs and methyl jasmonate (MeJA) on the growth of Arabidopsis thaliana seedlings (Col-0) was analyzed. The single application of 20 µM MeJA provoked a reduction in the fresh weight of the Arabidopsis seedlings; besides the treatments with 20 and 30 µM MeJA that inhibited the elongation in the main root. Co-treatments of MeJA with PAs showed that the exogenous application of MeJA-Put inhibited more severely the growth of the primary root as well as the gain in fresh weight in all the lines analyzed, including the mutant insensitive to MeJA *jar1-1*. Also the co-treatment PAs with MeJA were analyzed with a jasmonic acid sensing line (*35S::JAZ1-GUS*). A significant decrease was found in the reporter GUS staining in the sensor line *35S::JAZ1-GUS* below treatments and co-treatments with the putrescine and the MeJA in different plant zones, suggesting an increase of jasmonates. Summarizing, these data suggest a connection between MeJA and polyamines.

The role of the TRPV channel in osmobiosis of the tardigrade *Hypsibius dujardini*

Erendira Anai De la Torre-Gonzalez, Zyanya Mayoral-Peña, Fausto Arellano-Carbajal

Laboratorio de Genética Molecular y Ecología Evolutiva, Facultad de Ciencias Naturales, Universidad Autonoma de Queretaro, Carretera a Chichimequillas S/N, Ejido Bolaños, 76140 Santiago de Querétaro, Qro., (442)1921200 ext. 65334, fausto.arellano@uaq.mx.

Cryptobiosis is an ametabolic state that confers resistance to different hostile stimuli such as desiccation, freezing and hyperosmotic stress. Tardigrades exhibit cryptobiosis, which involve a tun state to reduce the surface of the body in order to avoid water loss. There are other changes that occur in this state, such as the accumulation of carbohydrates, lipids and some proteins like LEA and CAHS that are necessary to protect against cellular damage. The tardigrade cryptobiotic tun state confers resistance to different stress conditions, which are lethal to most organisms. Osmobiosis is a type of cryptobiosis but is induced by loss of water due to high external osmolality. In mammalian cells and invertebrates, the subfamily of TRPV channels, such as TRP4, are involved in signal transduction related to mechanical and osmotic stimuli. Organisms that are phylogenetically related to tardigrades are known to have TRP4 ortholog proteins. For instance, OSM-9 and OCR-2 in *Caenorhabditis elegans* and IAV and NAN in *Drosophila melanogaster.* However, the mechanism used to detect osmotic changes in order to induce osmobiosis in tardigrades remains unknown. To date, genomic sequencing of the tardigrade *Hypsibius dujardini* and the establishment of techniques like RNA interference (RNAi) and *in situ* hybridization have helped to establish *H. dujardini* as an emergent model organism. Thus, we explored if *H. dujardini* presents an ortholog of NAN and if this channel regulates the response to hyperosmotic stress prior to entering the tun state. We ran a multiple sequence alignment with NAN on the genome of *H. dujardini* and conducted a phylogenetic analysis with maximum parsimony and a Bayesian approach. We found that *H. dujardini* TRP gamma is the closest protein to NAN. We next designed a set of specific primers to PCR-amplify a 439 bp product that we will clone in a PCR4 TOPO vector, in order to generate double-stranded RNA-mediated RNAi. We determined that exposure to 0.1M NaCl induced a tun state in 98% of the tardigrades (n=45). We plan to microinject the double-stranded RNA-mediated RNAi in the gonads of the tardigrades in order to evaluate osmobiosis behavior and different morphologic parameters in the progeny of microinjected tardigrades**.**

NaTrxh is an essential protein in the pollen rejection response in *Nicotiana*, which reduces to S-RNase increasing its activity.

María Daniela Torres Rodríguez[1], Bruce McClure[2], Lilian González Segura[1] and Felipe Cruz García[1].
[1].- Departamento de Bioquímica. Facultad de Química, UNAM. Ciudad Universitaria, México, 04510.
[2]. Schweitzer Hall 112 Schweitzer Hall, University of Missouri. Columbia, MO 65211
E-mail: biol_daniela@yahoo.com.mx

Many plant species have self-incompatibility (SI) systems that enhance outcrossing by discriminating between "self" and "non-self" pollen and specifically rejecting self-pollen, by favoring outcrossing. SI helps maintain species' genetic diversity.

SI in *Rosaceae*, *Solanaceae* and *Plantaginaceae* response depends on S-RNase and SLF/SFB *S*-specific interactions; however, genetic studies show that other modifier genes (MG) unlinked to the *S*-locus, are also required to perform the pollen rejection response properly. *120K, NaStEP* and *HT-B* are MGs on the pistil side, and *cullin 1, SBP*, *MdABCF* and *NaSIPP* are MGs in the pollen side. To complete our knowledge about how the SI mechanism occurs, we have been studying to *NaTrxh* as another possible MG in *Nicotiana*.

NaTrxh is a type h subgroup II thioredoxin that is abundantly expressed in pistils of SI *N. alata,* but it is expressed at low levels in self-compatible *Nicotiana* species. Biochemical and cell biology results show that NaTrxh is secreted onto the pistil extracellular matrix, that it interacts with the S-RNase, and reduces it *in vitro*.

Here, we show that S-RNase reduction by NaTrxh considerably increases its ribonuclease activity. We tested whether the N- or C-terminal domains in NaTrxh are involved in the S-RNase activation by creating mutant proteins lacking each domain; our results show that the N-terminal domain is involved in this activation, which is relevant because the S-RNase ribonuclease activity is crucial for pollen rejection. We also give evidence that NaTrxh reduces a specific disulfide bridge on S-RNase. Now, we are focused in to determine the structural changes that allow the S-RNase activation after being reduced by NaTrxh, for that reason we are working in the crystallization of both reduced S-RNase and the S-RNase-NaTrxh complex.

We performed loss of function assays by generating transgenic plants that overexpress a mutant version of NaTrxh, called NaTrxh^{WSGPS}, which does not exhibits reducing activity. We tested the pollen rejection in *N. plumbaginifolia* X *N. alata* mutant hybrids and our results show that pollen tubes growth reaching the base of the style in SI crosses, which clearly indicates that reducing activity of NaTrxh is crucial for SI in *Nicotiana*.

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"Characterization of the function of long non-coding RNA *lncTATA* on the development of *Arabidopsis thaliana*"

Susana Isabel Vargas Camacho1, Jaime Apórtela Cortez2 y Catalina Arenas Huertero2. Posgrado en Bioprocesos, Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí1. Facultad de Ciencias, Universidad Autónoma de San Luis Potosí2. Diagonal Salvador Nava Mtz s/n, Zona Universitaria, San Luis Potosí., SLP. ahuertero@gmail.com2

The non-coding RNAs (ncRNA), are functional entities that doesn't encode to a protein, they have been related to various activities including the regulation of gene expression, as well as participation within the transcription machinery. The ncRNAs are classified by the length of the transcript, the long non-coding RNAs (lncRNAs), are molecules with a length larger than 300nt, intermediate RNAs (ImncRNAs) have a length between 50-300nt, while the RNAs of shorter length of 50 nt are called smallRNAs of which miRNAs are highlighted.

In the present work, the effect of the long non-coding RNA lncTATA on the TBP2 gene, a member of the TBP family (TATA-BINDING PROTEIN) which participates in the transcription initiation, is characterized. The function of joining the TATA boxes of the promoters of various genes, including the genes of microRNAs (miRNAs). It has also been found that TBP2 interacts with TGH, a protein involved in the processing and maturation of miRNAs, therefore plants deficient in the expression of this lncTATA showed defects in the development, size and morphology of *Arabidopsis thaliana* leaves. So this work shows how an lncRNA has an effect on the regulation of a new regulatory network for the development of leaves in *Arabidopsis thaliana.*

Proteomics and metabolomics for Southwest of Mexico from CICY

Aguilar-Hernández, V.[1]; Brito-Argáez, L.[2], Galaz-Ávalos RM[2] and Loyola-Vargas VM.[2]

[1]Catedrático CONACYT, Unidad de Bioquímica y Biología Molecular de Plantas, Centro de Investigación Científica de Yucatán, Mérida, México.
[2]Unidad de Bioquímica y Biología Molecular de Plantas, Centro de Investigación Científica de Yucatán, Mérida, México.

Proteomics and metabolomics are key emerging technologies that either identify or quantify proteins and metabolites of a wide variety of sample types and underpin to develop advanced plant science and biotechnology. To create proteomics and metabolomics facilities and provide collaborative support to Southwest of Mexico, we are setting a mass spectrometry laboratory at Yucatan Center for Scientific Research (CICY). We developed a targeted metabolomics pipeline by internal standards to absolute quantitation of small molecules such as promoter growth regulators. To advance in the analysis of low abundance proteins, we reduced ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) protein using protamine sulfate-based precipitation from either uninduced leaf explant or undergoing direct somatic embryogenesis in *Coffea canephora*. Protein identification and quantification will aid in deciphering altered proteins on induction of direct somatic embryogenesis that then results in forming somatic embryos at the explant, without the foundation of callus. Consolidation of those omics facilities could help us to conduct a systematic analysis of plant biology events using metabolomics and proteomics.

Keywords: proteomics, metabolomics, LC-MS/MS, somatic embryogenesis, *Coffea canephora*.

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"Conformational changes regulate the half-life of proteins of the Bcl-2 family:"

Luis Alberto Caro Gómez[1]; Jorge Luis Rosas Trigueros[2]; Edgar Mixcoha[3], José Luis Vique Sánchez[1], Claudia Guadalupe Benítez Cardoza[1]; and Absalom Zamorano Carrillo[1*]
1 Laboratorio de Bioquímica y Biofísica Computacional, ENMH, Instituto Politécnico Nacional,
2 Laboratorio Transdisciplinario de Sistemas Evolutivos, ESCOM del Instituto Politécnico Nacional,
3 CONACYT-Instituto Nacional de Psiquiatría, Ramón de la Fuente Muñiz.

*Corresponding author. Email: azamorano@ipn.mx Phone number: 57296000 Ext 55542

Apoptosis is a natural process required for the removal of redundant cells during development, potentially dangerous cells and those in senescence. Cell death dysregulation has been implicated in a variety of human diseases such as cancer, neurodegenerative disorders, and autoimmunity. This process is regulated by several proteins among them, those belonging to the Bcl-2 family. Members of this family are grouped according to their participation in the apoptotic mitochondrial pathway in pro- and anti-apoptotic proteins. The members of this family are characterized by the Bcl-2 homology (BH) domains, BH1, BH2, BH3, BH4, as well as an intrinsically disordered region (IDR) depicted as “flexible loop domain” (FLD), and a transmembranal (TM) region that anchors mitochondrial outer membrane (MOM). The main core is formed by BH1, BH2 and BH3 domains that interact with BH3 domain of other family members. The interaction between anti-apoptotic and pro-apoptotic proteins of Bcl-2 family exquisitely regulate cell death. The IDRs are rich in PEST regions (proline (P), glutamic acid (E), serine (S) and threonine (T)). The two main degradation pathways mediated by protein PEST sites are degradation of ubiquitin-proteasome and cleavage of μ-calpain. Some studies report that the half-life for Bcl-2 is around 24 hours and for Bcl-2A1 is 30 minutes.
In this work, we simulated by molecular dynamics the three-dimensional models of proteins Bcl-2 and Bcl-2A1 using OPLS-AA force field, NPT conditions, at 310 K for 100 ns. A comparative analysis between Bcl2 and Bcl-2A1 described the conformational changes at an atomistic level of each construct and the contributions of IDR and PEST regions in structure, dynamics, and function.

An essential dynamics was also performed to identify global collective movements of proteins which are crucial for the regulation of biological activity. These results indicate that Bcl-2 protein hides the regions where the PEST sites are located, avoiding the attack of proteases, while the Bcl-2A1 exposes them, explaining the Bcl-2's longer half-life in comparison with Bcl-2A.

Evaluation of the rol of Spo0B as limiting factor in the information processing and decision making of the sporulation system of *B. subtilis*

Nori Castañeda Gómez, Diana X. Sahonero Canavesi, José Carlos Ramón Hernández, Rafael Peña Miller y Rosa Ma. Gutiérrez Ríos. Instituto de Biotecnología, UNAM. Av. Universidad #2001, Col. Chamilpa, Cuernavaca, Morelos, México. C. P. 62210. Phone: (52 777) 329 16 00, (52 55) 56 22 76 00 Fax: (52 777) 3 17 23 88. E-mail: rmaria@ibt.unam.mx

It is common to observe that when an isogenic bacterial population is stimulated by a determinate signal, the individual cells in the population do not behave in the same manner. How a cell chooses which cell fate to follow, is still poorly understood. This behavior is easier to study in organisms capable of differentiating, such as the spore forming bacteria *Bacillus subtilis*. It has been demonstrated that when a *B. subtilis* population is induced to form spores, only a small proportion choses spore fate between other possible options such as miner cell, cannibal cell, etc.

Most of the components of the phosphorelay controlling spore formation initiation (e.g. *spo0F, kinA*) have been studied to test if they are the limiting factor for spore formation initiation, except for the phospho-transferase *spo0B*. Research developed in other groups as well as bioinformatic studies developed in ours, support the role of *spo0B* as a limiting factor. The main objective of this work is to elucidate if the expression of *spo0B* controls the proportion of cells that initiates the sporulation process. In order to test this, *spo0B, kinA*, and *spo0F* genes were cloned under an IPTG inducible promoter, Phyperspank, and inserted in *B. subtilis* genome in separate strains. Furthermore, the strain Phyperspank-*spo0B* is *Δspo0B*, to avoid background expression of native *spo0B*. Additionally, the reporter genes P*spoIIQ*-GFP (late sporulation) and P*spoIIA*-DsRed (early sporulation) were added to each of these strains. The use of fluorescent reporters allows the tracing and study of this phenomenon with technics such as flow cytometry and microfluidics. Preliminary results using microfluidics have suggest that the microenvironment generated inside different types of chip could determine the behavior observed in the cells, which correlates with data from literature. The following experiments will involve the use of a microfluidic device called mother machine that allows the analysis of the behavior at the single cell level.

The strains mentioned above will be evaluated by bright-field microscopy and proportion of cells initiating sporulation will be quantified. Transcript levels of these components will be measured with RT-PCR to validate their IPTG induced modulation. Finally, native *spo0B* transcript levels in distinct *B. subtilis* growing phases will be quantified by RT-qPCR to analyze its effects in sporulation.

Viral metagenomic comparative analysis of the deep-sea hydrothermal vents

Hugo G. Castelán Sánchez1 and Sonia Dávila Ramos[1]
[1] Centro de Investigación en Dinámica Celular, Instituto de Investigación en Ciencias Básicas y Aplicadas, Universidad Autónoma del Estado de Morelos, Cuernavaca,Morelos, Avenida Universidad 1001, Chamilpa, C.P. 62209. México, correo: sdavilar@uaem.mx tel. (777)3297020 ext 3276

Viruses are the most abundant biological entities in the biosphere, which have the ability to infect in the three domains of life Bacteria, Archaea, and Eukarya, which have been estimated at least 10 times more abundant than bacteria. (Mokili et al., 2012; Bolduc et al., 2012; de Cárcer et al., 2015). However, within the hydrothermal vents, few viruses have been isolated by traditional techniques, but one strategy for but a strategy to elucidate the structure of viral communities in environments is the metagenomics.

Viruses play an essential role in the biogeochemical cycles, also viruses facilitate the horizontal transfer of genes between bacteria, being a factor that impacts the evolution of prokaryotic communities (Anderson et al., 201).

Thus the goal of this study was to describe to structure viral communities and the main functions of genes present in viromes, from sediments and hydrothermal fluid form deep-hydrothermal vent located in different regions of world.

For this study, 10 metagenomes were taken from hydrothermal vents from different parts of the world, from which the raw data of NCBI SRA were downloaded. We observed that typical families in sediments and plumes are within the order *Caudovirales* (*Siphoviridae, Podoviridae, Myoviridae*), which have been commonly reported in deep marine as in other environments such as soils and freshwater, regarding to the sediments, they had different structure in particular by ssDNA virus, that were absent in plume of hydrothermal vent, in this case the ssDNA viruses were found as *Microviridae* and *Inoviridae*, both families include bacteriophages no-tail. Also we performed a functional analysis of the viral sequences of the hydrothermal vents, in order to assess the metabolic profile of viromes, in general an abundance in frequency of nucleotide in function of carbohydrates, amino acids and derivatives, RNA metabolism, protein metabolism, and

particularly phages, prophages, transposable elements, and plasmid.

IMPORTANT RIBOSOMAL MUTATIONS ARE REVEALED BY GENOMICS OF *Avibacterium paragallinarum*

[1]María Elena Cobos Justo, [1]Patricia Sánchez Alonso, [1]Norma Rojas Ruiz, [2]Erasmo Negrete Abascal, [3]Víctor Pérez Márquez, [1]Candelario Vázquez Cruz. [1]Centro de Investigaciones en Ciencias Microbiológicas. Instituto de Ciencias. Benemérita Universidad Autónoma de Puebla. Edificio IC11, 24 Sur y Av. San Claudio. C.U., Col San Manuel, CP. 72570. Puebla, Pue. Tel: 2222295500, Ext. 2536. [2]Carrera de Biología. Facultad de Estudios Superiores Iztacala. UNAM. Av de los Barrios no. 1. Los Reyes Iztacala, 54090 Edo. de México. 3BIOVETSA Tehuacán Puebla México. Email:ecobacilos@yahoo.com

Avibacterium paragallinarum (AVPG) is a bacterium that belongs to *Pasteurellaceae* family. It is the etiological agent of infectious coryza, a disease of the upper respiratory tract of birds. AVPG is considered an annoying microorganism because it needs strict and expensive nutritional conditions for its growth, making difficult its manipulation in laboratory work. Until a few years ago little was known about the genetic information of this bacterium, but nowadays, with DNA massive sequence technology there is a set of AVPG-genomes published in GenBank and others from unpublished collections. From that information it is possibly to know the gene content of AVPG bacterium and to propose the probable role in the pathogenicity mechanisms that are used to stay in the host and cause harm. The genome of ten strains of AVPG was sequenced by Illimina's technology. Reads were assembled with several software tools to get long scaffolds. The coverage was approximately of 50X and the gene annotation was made within rast prokaryotic genome annotation server. The results showed that AVPG genomes are very similar between them, there are conservation of essential metabolic functions and genes that code for factors of virulence, for example *cdtABC*, *avxABC*, and genes that encode for fimbrial proteins like F17 and type IV. Also was found that AVPG contains some genes not previously described. Some genes could be involved in the persistence of this bacteria within the host, and others to enhance the growth of the bacteria. The 16S-RNA sequences were analyzed and was found a deletion of approximately 10 bp in the stem-loop responsible for the assembly of the minor subunit of the ribosome with the major subunit. This type of deletions probably is related to the inefficient function of the ribosome and maybe it is related with the inefficient bacterial development observed. This model is similar to laboratory ribosomal *E. coli* mutants.

Secretome Prediction of Two *M. tuberculosis* Clinical Isolates Reveals Their High Antigenic Density and Potential Drug Targets.

Fernanda Cornejo-Granados, Lucia Zatarain-Barrón, Vito Cantu-Robles, Alfredo Mendoza-Vargas, Camilo Molina-Romero, Filiberto Sánchez, Luis Del Pozo-Yauner, Rogelio Hernández-Pando, Adrián Ochoa-Leyva,Departamento de Microbiología Molecular, Instituto de Biotecnología (IBT), Universidad Nacional Autónoma de México (UNAM), Cuernavaca 62210,México. Tel. 56 22 76 14

Corresponding author: Dr. Adrián Ochoa-Leyva aochoa@ibt.unam.mx

The Excreted/Secreted (ES) proteins play important roles during *Mycobacterium tuberculosis* invasion, virulence, and survival inside the host and they are a major source of immunogenic proteins. However, the molecular complexity of the bacillus cell wall has made difficult the experimental isolation of the total bacterial ES proteins.

Here, we developed a bioinformatics pipeline to predict the secretomes of two Beijing genotype *M. tuberculosis* clinical isolates obtained from patients from Vietnam (isolate 46), and South Africa (isolate 48) and the reference strain H37Rv. We observed that ~12% of the genome-encoded proteins are ES, being PE, PE-PGRS, and PPE the most abundant protein domains. Additionally, the Gene Ontology, KEGG pathways and Enzyme Classes annotations supported the expected functions for the secretomes. Notably, ~70% of an experimental secretome compiled from literature was contained in our predicted secretomes,while only the 34-41% of the experimental secretome was contained in the two previously reported secretomes for H37Rv.

These results suggest that our bioinformatics pipeline is better to predict a more complete set of ES proteins in *M. tuberculosis* genomes. Furthermore, the predicted ES proteins showed a significant higher antigenic density measured by Abundance of Antigenic Regions (AAR) value than the non-ES proteins and also compared to random constructed secretomes. Additionally, we predicted the secretomes for H37Ra, and two *M. bovis* BCG genomes and observed that the antigenic density for BGG and for isolates 46 and 48 was higher than the observed for H37Rv and H37Ra secretomes. Interestingly, mice infected with Beijing isolate 46 showed a significant lower survival rate than the ones infected

with isolate 48 and both survival rates were lower than the one previously reported for the H37Rv strain in the same murine model. Finally, after a druggability analysis of the secretomes, we found potential drug targets such as cytochrome P450, thiol peroxidase, the Ag85C, and Ribonucleoside Reductase in the secreted proteins that could be used as drug targets for novel treatments against Tuberculosis. This bioinformatics strategy was recently published in Frontiers in Microbiology (doi: doi: 10.3389/fmicb.2017.00128)

The genetic interaction landscape of long lived mutants in *Saccharomyces cerevisiae*

Erika Viridiana Cruz Bonilla, Alexander de Luna
Unidad de Genómica Avanzada (Langebio), Centro de Investigación y de Estudios Avanzados del IPN. Irapuato, Gto. México, CP 36824, +52 (462) 166 3000 ext. 3069
erika.cruz@cinvestav.mx, alexander.deluna@cinvestav.mx

Aging is characterized by the progressive accumulation of damage and eventual loss of cell function. With the aid of model organisms, genes affecting life expectancy have been identified, many of which are conserved across different species and taxonomic groups. However, little is known about how all these genes interact with each other to modulate lifespan; since most of these genes that alter lifespan have only been studied independently from one another. Understanding how genes modulating life expectancy interact with one another will help us establish how many different ways of extending cell longevity are there, which cellular processes are involved in determining life expectancy, and how can lifespan be further increased.

We will present a new research project to describe pairwise genetic interactions among knock-out mutants that increase lifespan in the budding yeast *S. cerevisiae*. We set out from a group of over 200 knock-out mutants that had previously been reported to increase the chronological lifespan of yeast. We will inquire into the mechanisms responsible for the increase in lifespan in these mutants with a database approach, integrating the genomic data from other phenotypes to propose which cellular processes may be altered in these mutants that could be responsible for their effect on aging. We will complement the findings of the database search with analysis of transcriptomes of the knock-out mutants.

In parallel, once the long-lived phenotypes of the mutants of interest have been confirmed, we will proceed to generate double mutants to generate a network of genetic interactions. From this network we will inquire the frequency of positive, neutral, or negative epistasis between genes of interest, which shows which genes might act on common or compensatory pathways. We will also cluster genes based on their connectivity and, in combination with GO terms, we will assign them to functional groups that will reflect which cellular processes are principally modulating lifespan in these mutants and how these functions act together to determine longevity.

Congenital absence of uterus: elucidation of the gene modules involved in the development of the female urogenital tract

Fernando Fernández[1], Atlántida Raya-Rivera[2], Ana Claudia Velázquez-Wong[3], Eva Martínez-Peñafiel[4], Alejandro Martínez-Herrera[1,5], Jonathan Zaiat[6], Alejandro Marmolejo-Valencia[7], Haydeé Rosas Vargas[3], Luis Kameyama[4], Marcelo Martí[6],Adrián Turjanksi[6], Horacio Merchant[7]

[1] Unidad de Genética, Hospital General de México.
[2] Laboratorio de Ingeniería de Tejidos, Hospital Infantil de México.
[3] Unidad de Investigación Médica en Genética Humana, CMN Siglo XXI.
[4] Departamento de Genética, CINVESTAV-IPN
[5] Facultad de Medicina, UNAM.
[6] FCEyN, Universidad de Buenos Aires-Bitgenia, Argentina.
[7] Instituto de Investigaciones Biomédicas, UNAM.

Dr. Balmis 148 Cuauhtémoc 06720, Ciudad de México. Tel. 27892000x5811
ffernandez@ciencias.unam.mx

Congenital absence of uterus and vagina (CAUV) affects 1:4500 women. The etiology of this condition remains unexplained, and the molecular basis of the female urogenital tract (FUT) development, including its gene regulatory networks (GRN), has been scarcely studied. Thus, we performed several approaches in order to identify genetic elements potentially involved in this morphogenetic system. (1) Mutational analyses in patients with CAUV, including genomic microarrays and exome sequencing, revealed candidate genes corresponding to the WNT signalling pathway, ESR transcriptional regulation, among others. (2)

Transcriptome analyses in murine mesenchymal embryonic tissues confirmed the activation of canonical WNT and TGF-beta signalling modules in females, and allowed the identification of additional olfactory receptors and G-protein coupled signalling elements. (3) Phenolog analyses, motif search on promoter regions, and functional interactions (STRING) analyses provided additional candidate genes.

Using these data we aimed to integrate a preliminary GRN of FUT development, and the achieved results support a gene interactions model for CAUV, and suggest that this condition may result from perturbations in the elongation phase of the Mullerian ducts, rather than on its differentiation phase. Aberrant AMH/Sp7 activity cannot be ruled out, and further studies including cell-specific transcriptome profiling and extended patient mutational analyses will provide a better understanding of the molecular mechanisms underlying CAUV. CONACYT-133273, UNAM-PAPIIT IN219912, CONICET 100 exomas.

Functional analysis of genes expressed during cell differentiation of *Sclerotium cepivorum* Berk

David Alberto García Estrada, Alberto Flores Martínez, Patricia Ponce Noyola
Departamento de Biología, División de Ciencias Naturales y Exactas, Campus Guanajuato, Universidad de Guanajuato
Noria Alta s/n; C.P. 36050; Guanajuato, Gto.; Phone. (+52) 01 473 732 006 Ext 8149; E-mail: poncep@ugto.mx, floralb@ugto.mx

Sclerotium cepivorum Berk is a specific phytopathogen of Allium genus plants, it causes “garlic white rot” (**Coley Smith, 1990**). Guanajuato and Zacatecas are the main states garlic producers and exporters in Mexico, this is an important justification for study of this disease and to design strategies for to eliminate the white rot (**SAGARPA 2010**). The resistance of this disease has been a big problem because this fungus form resistance structures called sclerotia (**Ferguson, 1953**). In our working group, was obtain a mutant strain (ScM) with alterations in its development, like delay growth in comparison with the wild strain (ScWT) and does form conidia instead of sclerotia. A genomic, transcriptomic and proteomic analysis of the *Sclerotium cepivorum* Berk strains, wild type and mutant, was carried out during cell differentiation. From the proteomic analysis, it was detected 69 differential soluble proteins between ScM and ScWT strains which are not registered in the UniProt server Database. We analyzed the promoter sequences of these genes coding for differential proteins using **TRANSFAC® PATCHTM** tool (**Volker Matys et al., 2006**). The results obtained were compared against Fungal Transcription Factor Database (FTFD) (**Park et al., 2008**). We obtained seven differential proteins meet the selection criteria established in the developed pipeline. To determine the role of these proteins during cell differentiation of *S. cepivorum* Berk, we cloned, overexpressed and purified two of these proteins.

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Identification of *trans*-acting riboswitches in bacterial genomes

Edgar Rodríguez García1 & Enrique Merino Perez1.
1Departamento de Microbiología Molecular, Instituto de Biotecnología - UNAM, Av. Universidad 2001,Cuernavaca, Mor, CP 62210, Mexico. Tel: 7773114904. Email: merino@ibt.unam.mx, rdzedgar@ibt.unam.mx

The ability of organisms to respond to certain stimuli, as well as to adapt to diverse conditions, involves cellular processes whose responses, mostly, need to be regulated by changes in genetic expression. This genetic regulation is carried out by elements that act at transcriptional, translational or post-transcriptional level. In a general way, most regulators are proteins that bind to DNA or RNA specific sequences, depending whether the regulation is at transcriptional or translational level; nevertheless, in some other cases, regulation can be mediated by RNA molecules.

For many years, there was the belief that the role of RNA was limited to participate in the central dogma of molecular biology as intermediary in proteins production. Nowadays, it is known that RNA is an extremely dynamic molecule with characteristics and properties that allow it to participate and modulate different molecular and cellular processes (Mattick, 2004)

One way in which RNA can modulate genetic expression is through riboswitches, elements with the ability to recognize its target molecules, which allows them to change its threedimensional conformation and modulate genes expression (Winkler, W. C., Cohen-Chalamish, S., & Breaker, 2002). A few years ago, the evidence showed that riboswitches

only acted in *cis*, that is, in the same sequence plane with near genes, however, Loh *et al*.in 2009, found a riboswitch called SreA, which senses S-adenosylmethionine (SAM) and is able to act both, *in cis* and *in trans*, in *Listeria monocytogenes*. As far as we know, this is the only case of a reported *trans*-acting riboswitch.

Nowadays, with the new massive sequencing technologies available to everyone, complete genomes are obtained everyday but, most of this information is used only to compare certain genome regions or to analyze particular genes, and all the other information is stored without getting a greater profit. The use of bioinformatics allows to analyze large amounts of data to verify biologically significant hypotheses and answer educated questions that lead to the generation of knowledge.

The present work is intended to analyze exhaustively bacterial genome sequences to identify new *trans*-acting riboswitches, different to the one reported by Winkler *et. al*.

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Behavior of the antimicrobial peptides Pin2 and Pin2GVG in membranes. Molecular Dynamics Simulations

Ramón Garduño-Juárez[1], José Luis Velasco-Bolom[2]
[1]Instituto de Ciencias Físicas, UNAM, Campus Morelos
[2]Programa de Doctorado en Ciencias Bioquímicas, UNAM ,Av. Universidad S/N, Col. Chamilpa, 62210 Cuernavaca, Morelos 01(777)3291749; ramon@icf.unam.mx

A possible mechanism of insertion, oligomerization and pore formation was detected in the antimicrobial peptide Pin2 and its analogue Pin2GVG when interacting in membrane models (POPC and POPE: POPG), the possible differences in their mechanism of action are postulated. Simulations on a mesoscopic scale allowed a broader sampling of the phenomenon, while atomic-scale simulations allowed us to access a better spatial resolution, but a broader sampling is almost impossible.

Most of our results reported this time focus on a detailed description of the pore formation process by Pin2 on POPC membranes. We conclude two important aspects of this phenomenon: the first is that our results indicate that Pin2 forms a pore of toroidal shape and not a pore in the form of a barrel as had been proposed in experimental studies. The second aspect is that the peptides have a preference to be oligomerize in an antiparallel manner, i.e. the N-terminal end of a peptide can interact with the C-terminal end of the adjacent peptide which can reinforce the idea of a cooperative effect for the insertion of these peptides. Although the parallel orientations also appear and form pores.

In the literature it has been reported that certain pore-forming proteins and peptides can be selective towards some type of ion, our results indicate that the pores formed by Pin2 are selective to the chlorine ion. The hydrophilic part of the peptides is oriented towards the center of the pore being determinant for the passage of ions and water molecules. The hydrophobic zone of the peptides interacts with the lipids of the membrane allowing a curvature of the same.

Furthermore, simulations with POPE: POPG lipid mixtures provide interesting results, due to the formation of lipid haloes around the peptide oligomer, this interaction can be mediated by electrostatic interactions of POPG phosphates.

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Characterization of the docking mechanism of peptide variants of Discrepin on the Kv4.3 potassium channel. Molecular Dynamics Simulations

Ramón Garduño-Juárez[1], Pavel Andrei Montero-Rodríguez[2]
[1]Instituto de Ciencias Físicas, UNAM, Campus Morelos
[2]Programa de Maestría en Ciencias Bioquímicas, UNAM, Av. Universidad S/N, Col. Chamilpa, 62210 Cuernavaca, Morelos 01(777)3291749; ramon@icf.unam.mx

Ion channels are membrane proteins present in all cells, these facilitate the permeability of different ions through the lipid bilayer. Discrepin is an α-toxin (α-KTx15.6) extracted from the venom of the venezuelan scorpion *Tityus discrepans* that affects the ionic transit in the voltage-dependent potassium channels (Kv) of type A current, as is the case of the Kv4 channel. 3. Given the modulating potential of Discrepin and given that potassium channels are related to many aspects of the CNS neuronal function, studying the action of Discrepin at the molecular level represents a great opportunity for the development of new drugs for the control of disorders, such as epilepsy, dementia, anxiety and depression.

This time we report the modeling of the pore-forming domain in the Kv4.3 channel and the interaction of these with the Discrepin mutants (K13A), (AK13) and (V6K, D20K) that have affinity for Kv4.3 and for which there is no experimental data of his Kd.

The homology modeling of the pore-forming domain in Kv4.3, made of segments S4-S5-S6, was made with MODELLER and Swiss-Model. The molecular docking between Discrepin and its peptide variants with the Kv4.3 channel were made with ClustPro.

Likewise, in this communication we report some results of the Molecular Dynamics runs of the Kv4.3/Discrepin (mutants) complexes from which it is intended to obtain Kd values by means of the calculation of the Potentials of Mean Force.

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Phylogenetic analysis of members of the MAP kinase family (MAPK) in Viridiplantae

José Manuel González-Coronel[1], Ángel Arturo Guevara-García[1]✉
[1]Instituto de Biotecnología, Universidad Nacional Autónoma de México. Dpto. Biología Molecular de Plantas. Av. Universidad 2001, Chamilpa, 62210 Cuernavaca, Morelos.
✉ To whom correspondence should be addressed. E-mail: aguevara@ibt.unam.mx. Contact number: +52 (777) 329-1777 ext 38134.

Protein phosphorylation operating in various signaling pathways is regulated by the activity of enzymes known as kinases. Among the different proteins with kinase activity are the so-called MAPKs (by Mitogen-Activated Protein Kinases), which are responsible for amplifying and transducing various intra and extracellular signals in order to mount an appropriate response for a specific stimulus. The MAP kinases modules consist of three different gene families named: MAPK or MPK, MAPK kinase (MKK or MEK), and MAPKK kinases (MPKKK or MEKK), whose components are activated sequentially by the phosphorylation of amino acids in the activation domain. Particularly, MAPKs are activated by the dual phosphorylation of the TxY motif by some member of the MKK family. The MAPKs cascades are ubiquitous in eukaryotic organisms and particularly in the plant lineage, they have been implicated in the control of multiple responses to biotic and abiotic stress conditions, as well as in development programs. As a starting point to establish the evolution of these proteins in the clade Viridiplantae, an *in silico* genomic analysis of the MAPKs from 14 plant species belonging to different lineages of the plant kingdom, ranging from unicellular green algae to plants with flowers, was performed, and a name was assigned to each of the predicted MAPKs based on their evolutionary relationship by orthogenesis with the *Arabidopsis* MAPKs. The phylogenetic analysis allowed to classify them into five different groups (A, B, C, D and E). In addition, the detailed study of protein sequences revealed the existence of domains located towards the N- and C-terminal ends, not previously described as distinctive of a specific group of MAP kinases. On the other hand, the analysis also revealed the existence of activation motifs different from the typical activation domains T-E-Y and T-D-Y characterizing canonical MAP kinases. The subsequent study of such motifs could result in novel information regarding potential targets of the MAPKs operating in the various signaling pathways in which they participate.

Key words: Plant Mitogen-Activated Protein Kinases (MAPKs), activation motive, conserved motifs, phylogeny.

A matrix-based stoichiometric model of *Helicobacter hepaticus* metabolism

Marco A. Gámez-Guerrero§, Alfredo Hernández-Ruiz§, José I. Hernández-Oropeza¶

Faculty of Engineering, Universidad La Salle México, Benjamín Franklin 45, Condesa, 06140, Mexico City, Mexico. Tel. +52 (55) 5278 9500 Ext. 2274.

§ These authors contributed equally to this work.
¶ Corresponding author. Email: jose.hernandez@ulsa.mx

Helicobacter hepaticus is a gram-negative, spiral-shaped microaerophilic bacterium [1]. *H. hepaticus* can lead to chronic hepatitis and hepatocellular carcinoma in certain strains of mice, as well as it has been recently demonstrated a pathogenic effect of *H. hepaticus* on human liver cells *in vitro* [2]. *H. hepaticus* is currently the best studied of the enterohepatic *Helicobacter* species [3]. There is evidence that in *H. hepaticus* proline serves as a key energy source and its metabolism can modify the redox environment that it affects pathogenicity *in vivo* for this gastrointestinal pathogen [1]. Besides, it is well known that urease is essential for colonization during *H. hepaticus* infection [3]. In this work, we propose a matrix-based stoichiometric model with the aim to give an insight into the metabolism of the emerging human pathogen *H. hepaticus*. To our knowledge, this is the first study in which *H. hepaticus* metabolism is represented mathematically. Identification of the metabolic network structure and application of the stoichiometric analysis were needed to build the model. The metabolic network structure includes 31 enzymes, 32 compounds and 32 intracellular reactions from 6 metabolic pathways: arginine and proline metabolism, alanine, aspartate and glutamate metabolism, citrate cycle, pyruvate metabolism, urea cycle and oxidative phosphorylation; information was taken from KEEG Pathway Database. Subsequently, we applied a modification of the algorithm by Mavrovouniotis [4] to the set of selected reactions. We theoretically synthesized a feasible, simple biochemical pathway formed by 13 simplified reactions by which *H. hepaticus* can produce urea, H2O2 and ATP from proline, aspartate and acetate in microaerophilic conditions. Finally, we set up the stoichiometric analysis. The stoichiometric matrix, $\boldsymbol{T}$T, representing the simple biochemical pathway has a dimension of 19 x 13 with a range of 11, which indicates the degrees of freedom of the system. Matrices $\boldsymbol{A}$1 and $\boldsymbol{A}$2 were calculated from $\boldsymbol{T}$T in order to find explicit expressions to quantify all internal metabolic fluxes. The conditional number of every matrix is less than 10; therefore, the model is not sensitive. The biochemical information available in the KEEG Pathway Database allows the reconstruction of a consistent metabolic network, which is fundamental in quantitative studies of cellular physiology. Through stoichiometric analysis is possible to determine the implicit constraints in metabolic networks. The stoichiometry of a biochemical system restrain the metabolic coefficients within a biologically meaningful interval, providing reliable understanding of the microbial metabolism, this information could be helpful for drug discovery and therapeutic target identification as a more rational approach.

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Cracking the mysteries of zygotic embryogenesis in avocado by systems biology

Janet Juarez-Escobar[1], Olinda Velázquez López[1], Monica Ramírez Vázquez[1], Lorena López-Sánchez[1], Jose M. Elizalde-Contreras[1], Juan L. Monribot-Villanueva[1], José A. Guerrero-Analco, Víctor M. Loyola-Vargas[2], Martín Mata-Rosas[3], Eliel Ruiz-May[1]

[1]Red de Estudios Moleculares Avanzados y [3]Red de Manejo Biotecnológico de Recursos, Clúster Científico y Tecnológico BioMimic®, Instituto de Ecología A.C. (INECOL), Xalapa, Veracruz, México. Tel. 228 8421800 ext. 6406 Email: martin.mata@inecol.mx y Email: eliel.ruiz@inecol.mx
[2]Unidad de Bioquímica y Biología Molecular de Plantas, Centro de Investigación Científica de Yucatán, Mérida, Yucatán, Mexico.

Angiosperm embryogenesis is a process that begins right after single or double fertilization with the formation of a zygote, that after several cellular divisions and differentiation will become a mature embryo. In general, embryogenesis involves the morphogenic transition through the establishment of a basal cell, a suspensor, and the globular, oblong, heart, torpedo and cotyledonary stage. The understanding of the process of embryogenesis at the structural, biochemical and molecular level forms the basis for comparison with other types of embryo formation, such as somatic, pollen, adventitious embryogenesis as well as the parthenogenetic embryo. However, in avocado, little is known about the transition through the canonic stages of embryogenesis process; the knowledge of it would be useful for improving the current protocols used for *in vitro* regeneration via somatic embryogenesis in avocado, given that the low rate of somatic embryos conversion is the current main bottleneck of this process. The primary goal of this study is to integrate structural features of zygotic embryogenesis acquired with advanced microscopy and massive proteomics and metabolomic massive data. As the first stage in this study, we present preliminary information on confocal microscopy and proteomics data during the zygotic embryogenesis of *Persea americana* Mill var. Hass. The results would give us the insights of avocado development stages and the establishment of a pattern for further determination of the way in which nutrients, hormones, metabolic pathways and other *in vitro* culture factors affect this developmental process.

Keywords: avocado, zygotic embryogenesis, advanced microscopy, proteomics, metabolomics

Metabolic role of aldehyde dehydrogenases in Pseudomonas aeruginosa PAO1

Adriana Julián-Sánchez[1], Gabriel Moreno-Hagelsieb[2], Rosario Muñoz-Clares[3], Héctor Riveros-Rosas[1,2]
[1] Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México, CdMx, 04510, México. [2] Department of Biology, Wilfrid Laurier University, Waterloo, ON , N2L 3C5, Canada. [3] Departamento de Bioquímica, Facultad de Química, Universidad Nacional Autónoma de México, Ciudad de México, CdMx, 04510, México.
hriveros@unam.mx; adh@bq.unam.mx; Tel +52 (55) 5623 2284

Pseudomonas aeruginosa is a Gram-negative and ubiquitous bacterium. It is a metabolically versatile opportunistic pathogen that normally inhabits the soil and surface water, and is capable of causing a wide array of acute and chronic infections. *P. aeruginosa* PAO1 possesses a large genome (encoding 5567 genes), lending to its adaptability to varying environments. Its metabolic capacity is extensive as exemplified by their ability to produce multiple secondary metabolites, as well as their ability to use a broad range of substrates. The repertoire of *P. aeruginosa* genes, which are substantially conserved, is consistent with the highest proportion of regulatory genes and networks observed in known bacterial genomes and is foundational to respond and adapt to diverse environments. *P. aeruginosa* PAO1 possesses an unexpected high number (23) of aldehyde dehydrogenase (*aldh*) genes (by comparison humans possess only 19 different *aldh* genes), but only a few these ALDHs have been characterized. To obtain insights about the metabolic role performed by each one of the 23 ALDHs found in *P. aeruginosa* PAO1, the genomic neighborhoods of *aldh* genes were analyzed and compared with neighborhoods in other *Pseudomonas* strains. Thus, phylogenetic analyses and genomic context data of *aldh* genes were used to predict the functional role of ALDHs in *P. aeruginosa* PAO1. Obtained results showed that found ALDHs belong to ALDH families 3, 4, 5, 6, 7, 8, 9, 11, 14, 18, and 27, as well as 6 additional families not named yet by the ALDH nomenclature committee. These ALDHs seem to be involved in several metabolic roles such as betaine and proline synthesis, beta alanine, glyceraldehyde 3-phosphate, and succinate semialdehyde metabolism, propanoate and amino acids catabolism, among others.

It is interesting to note that *P. aeruginosa* PAO1 possesses several ALDH isoenzymes that belong to the same family. Thus, three different *aldh* genes from *P. aeruginosa* PAO1 belong to ALDH6 or to ALDH27 families, and two *aldh* genes belong to ALDH14 family. This last, as well as the genomic context of these genes suggest that different ALDH isoenzymes are used to challenge different metabolic conditions, which might contribute to the ability of this bacterium to survive and adapt to varying environments.

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IDENTIFICATION AND ANALYSIS OF THE GENES INVOLVED IN THE BIOSYNTHETIC PATHWAYS OF CELLULOSE AND LIGNIN IN *Agave tequilana* Weber

Luis Fernando Maceda López[1], June Kilpatrick Simpson Williamson[2], Emmanuel Ávila de Dios[2], Elsa Beatriz Góngora Castillo[3], Fulgencio Alatorre Cobos[1§].

[1]Colegio de Postgraduados Campus Campeche. Carretera Haltunchén-Edzná km 17.5, Sihochac, municipio de Champotón, Campeche. C. P. 24450. MÉXICO, Camp.
[2]Unidad de Biotecnología, Centro de Investigación Científica de Yucatán, Calle 43 No. 130, Col. Chuburná de Hidalgo, 97070 Mérida Yucatán, México.
[3]Centro de Investigación y Estudios Avanzados del IPN –Irapuato. Libramiento Norte Carretera Irapuato León Kilómetro 9.6, Carretera Irapuato León, 36821 Irapuato, Gto.

[§]Corresponding autor e-mail: fulgencio@colpos.mx

The biosynthetic genes in primary and secondary cell wall formation have been mostly explored in dicotyledonous plants, especially for the herbaceous plant *Arabidopsis thaliana*. Likewise in monocotyledonous plants, few elements of cell wall biosynthesis routes have been reported and the interaction between them is unknown. The objective of this study is to identify and characterize the orthologous genes involved in cellulose and lignin biosynthetic pathways that are related with the cell wall formation in *Agave tequilana* Weber. For this purpose, were used in this work the unpublished transcriptomes are from different tissues of *Agave tequilana* Weber: anther, leaf, ovaries, stem, pistils, root, meristem and tepales, from Dr. Junes Simpson's laboratory (CINVESTAV Irapuato). Perfoming a data mining approach, a strategy that allows the analysis of genomic data and gene discovery, we will reconstruct for the first time the biosynthesis pathways of lignocellulosic compounds of the cell wall in *Agave tequilana* Weber. Subsequently, for the experimental analysis of the expression of some candidate genes identified *in silico*, qRT-PCR assays for *Agave tequilana* Weber and *Agave fourcroydes* Lem.

Key words: Transcriptome, Orthologous genes, Cellulose, Lignin.

Identification of a group of microRNA's involved in the progression of breast Cáncer

A.D.- Martínez Gutiérrez*; C.-Pérez-Plasencia*
*Laboratorio de Genómica, Instituto Nacional de Cancerología, Av. San Fernando No. 22, Col. Seccion XVI, México, D. F., C.P 14080, Tel. 56-28-04-00, email: carlos.pplas@gmail.com

ABSTRACT:

MicroRNAs are known to be deregulated and implicated in cancer progression and are proven to be useful as biomarkers. Recent evidence suggests the existence of upregulated microRNA's or oncomir's in breast cancer, although it's clinical importance hasn´t been well established. In this study we hypothesize and identify the existence of key driver oncomir's which are upregulated in every clinical stage of breast cancer, regulating key events implicated in tumour progression and with clinical importance. We comprehensively analyzed 1054 breast tumour microRNA profiles from The Cancer Genome Atlas (TCGA). We first compared normal versus tumour profiles, the resulting differentially expressed miRNA were further correlated with the tumour stage. Top overexpressed miRNA's with a positive correlation coefficient were used for the survival analysis. Cox regression analysis showed that 4 miRNA's (hsa-miR-340-3p, hsa-miR-877-5p, hsa-miR-940, hsa-miR-1307-3p) were able to predict the overall survival of the TCGA cohort. In addition, the predictive potential of these four miRNA's was further validated in two independent cohorts (METABRIC and GSE4267). Putative targets were identified using the top 5 prediction algorithms, we filtered only those mRNAs that were downregulated in tumour samples and who had a negative Pearson correlation with their corresponding microRNA. In summary our results describe four miRNA's involved in breast cancer progression.

Comparative genomics analysis of seven Mexican strains of *Anaplasma marginale*, the first *Anaplasma* genomes reported in Mexico

Fernando Martínez Ocampo, Rosa Estela Quiroz Castañeda, Edgar Dantán González
Unidad de Anaplasmosis, Centro Nacional de Investigación Disciplinaria en Parasitología Veterinaria (CeNID-PaVet), Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Carretera Federal Cuernavaca Cuautla 8534, Col. Progreso, C.P. 62574 Jiutepec, Morelos, México.
Tel: (52) 777 320 55 40 Ext. 128. E-mail: re quiroz79@yahoo.com.mx

Anaplasma marginale is a non-culturable intraerythrocytic bacterium that infects cattle and some wild ruminant animals. This bacteria is widely distributed worldwide, especially in tropical and subtropical regions and is the main etiologic agent of bovine anaplasmosis. The clinical signs of bovine anaplasmosis are progressive hemolytic anemia, fever, excessive weight loss, loss of milk production, abortions, jaundice, and even sudden death. The analysis of *A. marginale* genomes is a safe and effective alternative to identify peptides or proteins that have the antigenic and immunogenic potential predicted to prevent bovine anaplasmosis. Currently, only 15 genomes of ten American and two Australian strains of *A. marginale* have been deposited in GenBank database.
Recently, CeNID-PaVet, INIFAP isolated seven Mexican strains of *A. marginale* from different geographic regions of Mexico. The aim of this work is to genomes assemble and compare genomic features between the seven Mexican strains and identify the relationship with American and Australian strains of *A. marginale*. Furthermore, identification of genes involved in pathogenicity and antibiotic resistance has a relevant interest.
In this work, we report the seven draft genome sequences of *Anaplasma marginale* Mexican strains named as INIFAP01 to INIFAP09, which consist of ~1.17 Mb total length. In total, between 32 and 46 contigs were produced using *de novo* assembly with SPAdes (version 3.11.1) program, with N50 contig size between 61 and 85 Kb, G+C content of ~49.79%, and between 19 and 154X coverage. Phylogenetic analyses with 16S rRNA genes showed that the seven Mexican strains cluster with *A. marginale* species. Pangenome analyses showed that INIFAP01 (Tizimín, Yucatán) and INIFAP05 (Tlapacoyan, Veracruz) are related to American strains, and INIFAP02 (Texcoco, estado de México), INIFAP03 (Veracruz, Veracruz), INIFAP06 (Atitalaquia, Hidalgo), INIFAP08 (Puente de Ixtla, Morelos) and INIFAP09 (Aguascalientes, Aguascalientes) are related to Australian strains. The seven draft genomes were automatically annotated with RAST server, identifying between 1,178 and 1,218 genes, between 1,138 and 1,178 coding sequences (CDS), three rRNA and 37 tRNA genes. The automatic annotation showed that ~400 CDS are hypothetical proteins for each strain. Also, between 17 and 18 proteins were grouped in the virulence, disease and defense processes,and adhesins, toxins, superantigens and bacteriocins were not identified by RAST server.

MicroRNA regulation of homoeolog genes in polyploid hybrid species

Javier Israel Montalvo-Arredondo, Cei Abreu-Goodger
Unidad de Genómica Avanzada (Langebio), Cinvestav, Km 9.6 Libramiento Norte
Carretera Irapuato-León, Irapuato, GTO, México.
tel: +52 462 166 3000, email: {javier.montalvo,cei.abreu}@cinvestav.mx

Successful hybrids produced by species interbreeding, can present desirable characteristics that the parental species do not fully have, such as faster growth or resistance to environmental stress. There are many applications that benefit from the use of hybrids, including the “lager” yeast used by the beer industry and carps used in aquiculture. Artificial production of hybrids is generally very difficult to achieve for vertebrates, because of genomic incompatibilities during segregation that affect the viability of the offspring. In nature, the solution seems to rely on a total or partial duplication of the genome, generating polyploid but stable hybrid species. Orthologous genes that are brought into the same genome by hybridization events are referred to as homoeologs [1], and the unbalanced or lethal interaction of these homoeologs can affect viability. We think that successful hybrid species probably use different regulatory mechanisms to maintain balanced homoeolog expression.

Chen and co-workers propose that the microRNA regulatory system can provide stability at the level of a gene regulatory network (GRN) [2]. They observed that the higher the number of microRNA targets, the more stable the GRN becomes. We asked if this regulatory system could be involved in stabilizing homoeolog expression in polyploidy hybrids. As a model, we selected the tetraploid hybrid *Xenopus laevis*, categorized its genes, predicted microRNA targets, and analyzed a set of RNA-seq libraries of different tissues and developmental stages. Our results show that homoeologs are more likely to be predicted targets than genes that have lost their homoeolog pair. In addition, homoeolog pairs that are regulated as a couple by at least one microRNA family have more similar expression levels than pairs that do not share a regulatory microRNA. Thus, we think homoeolog pairs that were aided by microRNA regulation achieved higher stability and were retained more frequently. Differential gene expression analyses comparing oocyte

and early fertilization suggest that miR-34 could be balancing the homoeolog expression of key genes after fertilization. This microRNA could be of interest as a molecular tool or marker for the artificial generation of successful interspecies hybrids.

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Promiscuity of Histone acetyltransferases in *Ustilago maydis*

Jazmin Eliana Murcia Garzón1, Alfonso Méndez Tenorio[1], Juan Manuel González Prieto[2]

[1]. Laboratorio de Bioinformática y Biomedicina Molecular. Escuela Nacional de Ciencias Biológicas-Instituto Politécnico Nacional.
[2]. Biotecnología Vegetal. Centro de Biotecnología Genómica-Instituto Politécnico Nacional. Reynosa Tamaulipas.
***Correspondencia: correo postal: 11340 Teléfono: +52 (55)57296000 ext.62570. Correo:** jazelimurgar@gmail.com

The domains of proteins are considered as evolutionary and functional units, and a core of different interactions with the DNA and other proteins. Some domains are considered promiscuous, because they have ability to organize with several domains in the genome. These domains are important because they give structural and functional versatility to the proteins. An example of proteins with high versatility are histone acetyltransferases (HAT).

HAT are chromatin remodeling enzymes that acetylate the residues of lysine located mainly in tail of the histones. HAT are classified in eight families based on the analysis of motifs and domains: Gcn5/PCAF, Hat1, Hpa2/Hpa3, TafII250, Nuclear Receptor Coactivators, Elp3, CBP/p300 and MYST.

The high diversity of sequence and functional level of the different HAT families are indications the these enzymes have undergone different evolutionary processes of which there is scarce information. Therefore, it is necessary to clarify the relationship between the presence of promiscuous domains, with the functional diversity of these enzymes in *Ustilago maydis.*

In this work, we determine the organization of domains (architecture of the proteins) of the proteins of *U*. *maydis* obtained by HMM profile. Next, the domain pairs (bigram) were built from the architectures, and the frequency index of weighted bigram was determined by means different scripts developed by our research group.

By means of HMM strategy, we determine 5,668 architectures in proteome of *U*. *maydis*. Our method to determine promiscuity reveal that in *U*. *maydis* most families of HAT present several promiscuous domains, among which are the kinase, RING, PHD, bromodomain, and ZnFC2H2 domains. These domains will be targeted for the analysis of domain and protein interaction networks.

Secret-AAR: A Web Server to Assess the Antigenic Density of Proteins and for the Analysis of *M. tuberculosis* and *T. solium* Secretomes.

Adrián Ochoa-Leyva[1], Fernanda Cornejo-Granados[1], Juan Manuel Hurtado-Ramírez[1], Rogelio Hernández-Pando[2]

[1]Departamento de Microbiología Molecular, Instituto de Biotecnología (IBT), Universidad Nacional Autónoma de México (UNAM), Cuernavaca 62210, México. Tel. 56 22 76 14
[2] Experimental Pathology Laboratory, Department of Pathology, National Institute of Medical Science and Nutrition "Salvador Zubirán", Mexico City 14080, México. Tel. 55 54 87 09 00

Corresponding author: Dr. Adrián Ochoa-Leyva aochoa@ibt.unam.mx

The secretome refers to all the Excreted/Secreted (ES) proteins of a cell.

These proteins are involved in critical biological processes, such as cell-cell communication, signal transduction and modulating the host immune response.Recently, developed a bioinformatics strategy and published the most comprehensive experimental and predicted secretomes for two important human pathogens, *Mycobacterium tuberculosis* and *Taenia solium* (doi: 10.3389/fmicb.2017.00128), and we introduced the Abundance of Antigenic Regions (AAR) value to assess the antigenic density of a protein and to evaluate the antigenic potential of predicted and experimental secretomes (doi: 10.1038/srep09683). Now, to facilitate the use and acces to this tools, we implemented a user-friendly web server harvesting local CPU resources: Secret-AAR. It allows to i) calculate the AAR at genome-scale of proteins from any organism and ii) identify if a protein belongs to the secretomes of *Mycobacterium tuberculosis* and *Taenia solium*.

This web server can be a useful tool for researchers working on immunoinformatics and reverse vaccinology, aiming at discovering candidate proteins for new vaccines or diagnostic tests, and it can also help prioritize the experimental analysis of proteins for druggability assays. The Secret-AAR web server along with examples and tutorials is available at http://microbiomics.ibt.unam.mx/tools/aar/.

Identification of the immunodominant peptides of the PE_PGRS33 protein of *Mycobacterium tuberculosis*

David Ortega-Tirado[1], Aldo Arvizu-Flores[1], Carlos Velázquez[1], Clara Espitia[2], Adriana Sumoza-Toledo[3], Adriana Garibay-Escobar[1*]
[1]Departamento de Ciencias Químico Biológicas, Universidad de Sonora.
[2]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México. 3Instituto de Investigaciones Médico-Biológicas, Universidad Veracruzana.
[*] Departamento de Ciencias Químico Biológicas, Universidad de Sonora, Rosales y Luis Encinas s/n, 83000 Hermosillo, Sonora, México. Tel: (662) 2592163.
E-mail: adriana.garibay@unison.mx

Tuberculosis (TB) is a disease caused by *Mycobacterium tuberculosis* (*Mtb*) and it is the leading cause of mortality due to an infectious agent. TB cannot be efficiently controlled because there is no an efficient vaccine, since BCG is the only one authorized againts TB. Therefore, the development of a more efficient vaccine is necessary.

One of the current approaches in the vaccinology field is the development of peptide vaccines. The goal of this approach is to identify B or T-cell peptides that can elicit a protective immune response. These peptides comes from immunogenic proteins of the pathogen that can be identified by bioinformatic tools. In the case of *Mtb*, the PE_PGRS33 protein has been proved to be immunogenic. Therefore, the aim of this study was to identify the immunodominant peptides of PE_PGRS33 through bioinformatics. First, we employed the SYFPEITHI algorithm to make a prediction of the PE_PGRS33 T-cell peptides with the highest probability of being recognized by the MHC class II (IA^k molecule) and we selected five peptides based on their scores. Then, in order to increase the accuracy of the previous predictions, we built homology models for every peptide with MOE software, using the crystallographic structure of IA^K along with the immunodominant peptide of the hen egg white lysozyme (D Y G I L Q I N S).

The homology model results showed that two out of the five peptides (D V I N A P A L A[1], D G G I L I G N G[2]) resemble the interactions of the template peptide with IA^k. Both peptides have Asp in the first position, which enables peptides to establish important hydrogen interactions with Arg80 and Arg79 in the IA^k cavity. This cavity preferably requires that peptides have Ile in the fourth position, as in peptide 2 whereas peptide 1 have Asn that is also frequently to find in some peptides. Therefore, both peptides 1 and 2 are capable of establish contact with Tyr35 and Asn89 of IA^k. Both peptides are able to make hydrophobic contact with Phe38 in the cavity. In addition, both are capable to interact with Asn96 and His95 in IA^k just as the template peptide in the ninth position. The above results tell us that both peptide 1 and 2 are likely to be recognized by MHC II and being presented to T-cells.

"Functional Genomics of Germinating Sclerotia of *Sclerotium cepivorum* Berk"

John Dogulas Palleti1, Alfredo Herrera Estrella[2], Patricia Ponce Noyola[1]
[1]. Departamento de Biología. Universidad de Guanajuato. Noria Alta S/N. Col. Noria Alta, C.P. 36050. Guanajuato. México. Tel: +52(473)732006 Ex 8149. E mail: poncep@ugto.mx
[2]. Laboratorio Nacional de Genómica para la Biodiversidad, Centro de Investigación y de Estudios Avanzados del IPN Sede Irapuato, Irapuato 36821, Guanajuato, México. E mail: alfredo.herrera@cinvestav.mx

Sclerotium cepivorum Berk is the causative agent of the most destructive garlic disease called white rot. It attacks leaves, roots, and bulbs of garlic, and other Allium species throughout the world. *S. cepivorum Berk* produces highly resistant asexual reproductive structures (sclerotia) that can remain dormant for more than 15 years in the field and make the control of the fungus extremely difficult (Crowe *et al*., 1993). Sclerotia remain dormant until the fungus detects a potential host. However, there is scarce molecular information on the process of sclerotial germination.

To identify genes differentially expressed during sclerotial germination, we set up experiments to establish the transcriptional profile of the fungus in three conditions: dormant, activated and germinated sclerotia. Here, we report the transcriptome analysis using a total of 404,187,468 paired-end sequencing reads generated with the (Illumina® NexSeq500) platform. De novo transcriptome assembly was performed using Trinity®, producing a total of 25,205 transcripts with an average contig size of 1766 bp and a N50 of 3039. Mapping and annotation was carried out using the Blast2GO platform. Data were normalized using count matrix and filtered by TPM to identify differentially expressed genes using the Bioconductor framework. We used the Blast2GO results to identify the functional categories that are significantly enriched in the gene clusters obtained based on gene expression profiles to uncover relevant genes involved in the sclerotial germination process such as filamentous growth, fungal type vacuole, pathogenesis, peptide secretion, cell cycle, mRNA binding, chromatin assembly, and metabolism etc. We assessed and validated nine genes using RT-qPCR that are much more highly expressed in both germination conditions under study. These findings offer valuable information that lay a foundation for further genomics studies on this and related fungal species.

Transcriptomic analysis during the interaction of *Clavibacter michiganensis* subsp. *michiganensis* with two tomato species.

Leonardo Isaac Pereyra-Bistraín*, Cesaré Moisés Ovando-Vázquez±, Rosalba Castillo-Collazo***,** Dulce Alejandra Rougon-Cardoso+ and Ángel Gabriel Alpuche-Solís*
*****División de Biología Molecular, IPICYT, S.L.P.; ±Centro Nacional de Supercómputo, IPICYT, S.L.P.; +Escuela Nacional de Estudios Superiores, ENES UNAM, Gto.
Corresponding author: alpuche@ipicyt.edu.mx

Bacterial canker of tomato, caused by the actinomycete *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*), is one of the most important bacterial diseases of this crop, causing important economic losses worldwide, including Mexico. Unfortunately, there are no effective control strategies and only few preventive methods work efficiently. Currently, several wild tomato species such as *Solanum arcanum*, *Solanum habrochaites* and *Solanum pimpinellifolium* have been described with different resistance levels to *Cmm*, being *S. arcanum* LA2157 the most resistant species. However these are not commercially species. The study of these tomato wild species represents an important approach for identifying a natural resistance source that can be used in commercial tomato crops (*Solanum lycopersicum*) for controlling bacterial canker. Therefore, the aim of this work is to identify tolerance-related genes to *Cmm* by comparing the transcriptomic profile obtained with RNA-Seq of *S. arcanum* LA2157 (resistant) and *S. lycopersicum* (susceptible) during their first 24 hours of interaction with the *Cmm* pathogen. Our preliminary results suggest that both tomato species exhibit a high amount of differentially expressed genes from 0 hours to 24 hours after the *Cmm* challenge, being the 8 hours the most pronounced change. Particularly in the wild species *S. arcanum* LA2157, we found several enriched GO terms such as serine-protease activity, endonuclease activity, proteolysis and others potentially terms related to defense response; meanwhile, in the commercial tomato species these groups were not enriched. The future work relies on the validation of the expression levels of potentially tolerance-related genes to *Cmm* and to analyze them by virus-induced gene silencing in wild tomato species and finally to overexpress the candidate genes by gene editing (CRISPR/Cas9).

Halophile adaptations and stress in the lignocellulolytic fungus *Aspergillus sydowii*

Yordanis Pérez-Llano[1,2], Ramón A. Batista-García[1], Jorge L. Folch Mallol[2], María del Rayo Sánchez Carbente[2]
[1]Instituto de Investigación en Ciencias Básicas y Aplicadas, [2]Centro de Investigación en Biotecnología – Universidad Autónoma del Estado de Morelos
Address: Av. Universidad 1001. Col Chamilpa. Cuernavaca, Morelos. CP 62209.
Telf: 777 329 7000 ext 4550
e-mail: maria.sanchez@uaem.mx

The isolation of the halophilic strain of *Aspergillus sydowii* H1 is the result of the search for model microorganisms that possess two distinctive characteristics: the ability to grow in extreme conditions and the capacity to degrade plant biomass under these restrictions. The search for fungi that meet these requirements arises from the need to find enzymes with novel functionality and possible industrial applications. Interestingly, this strain can grow on diverse lignocellulosic materials, as well as on polyciclyc aromatic hydrocarbons, both on halophile conditions. To our knowledge, there is none information about the strategies that the fungus use to contend with high salinity. Therefore, we aimed to characterize the transcriptomic profiles of this strain to salinity when growing in wheat straw as sole carbon source. For this purpose, we characterized the growth of *A. sydowii* in a semi-solid fermentation of wheat straw in the presence of 0.5M and 2.0M of NaCl or in its absence. Some enzymatic activities involved in the breakdown of the lignocellulose (i.e cellulose, xylanase, esterase, phenol-oxidase and peroxidase) were assayed to determine the profile of secreted enzymes in the time course of the experiment. We chose to perform a transcriptomic analysis by RNA-Seq at day seven of growth. The main biological processes affected during growth in salinity conditions, as shown by GO term enrichment for the set of differentially expressed genes, are: organization, assembly or biogenesis of the cell wall, transport of cations and organic compounds through the plasma membrane, as well as processes of central metabolism. The main mechanisms of tolerance to salinity described in other microorganisms are present in *A. sydowii*, although with some particularities. Here we also delineate the mechanisms that are acting upon exposure to salinity and those that are particular to stress in salinity conditions, which are sometimes equally regarded to as mechanisms of halophilia.

Identification of co-expressed long non-coding RNA structural domains in similar human tissues.

Luis Jordan Perez-Medina and Selene Lizbeth Fernández-Valverde
Unidad de Genómica Avanzada-Langebio, CINVESTAV
Libramiento Norte Carretera Leon Km 9.6, S/N, 36821 Irapuato, Guanajuato, Mexico.
Phone: +52 462 166 3034
Email: selene.fernandez@cinvestav.mx

Gene regulation and subsequent differential gene expression is fundamental to the establishment of cell type and tissue identity. Over 200 cell types and nearly a hundred tissues have been described in humans. Long non-coding RNAs (lncRNAs) are RNA molecules over 200 nucleotides long that are generally not translated into proteins. lncRNAs have been shown to play roles as gene regulators through their interaction with proteins, DNA and/or RNAs. Some of these regulatory roles and binding capabilities are attributed in part to their ability to fold into complex tridimensional structures. Despite their intriguing functions, the vast majority of lncRNAs remain functionally and structurally uncharacterized. In this work, we identified gene co-expression modules comprised of coding and non-coding genes in 72 selected human tissues using publicly available data from the FANTOM-CAT project. We extracted 4,177 lncRNAs which belonged to 28 co-expressed gene clusters and predicted their secondary structures using NoFold, an approach which compares with previously known structures as annotated in the RNA structure database RFAM. We further identified structural families of lncRNAs which may be relevant to the establishment and maintenance of tissue identity by comparing them between tissues of similar characteristics. Finally, we tested whether any of the identified structural modules share binding with known RNA binding proteins. This project has allowed us to identify lncRNA structural families of interest as well as constituting a blueprint for similar studies in other less studied organisms.

Extremophile fungi: an alternative for the bioremediation of wastewater from municipal slaughterhouses in Morelos

Ing. BQM. Verónica Ramírez Lona
Dr. Ramón Alberto Batista García
Universidad Autónoma del Estado de Morelos
CP 62209 Av. Universidad 1001, Col. Chamilpa. Tel: (777) 329 70 20, rabg@uaem.mx

Currently, the growing pollution of water bodies is a problem that requires a lot of attention worldwide. Most of the slaughterhouses in Morelos state do not have a hygienic waste collection system. The blood collected is considered and treated as industrial waste, increasing treatment and operational costs. In this context, the Apatlaco river watershed is one of the water systems severely affected by this activity. Bioremediation is an environmentally friendly and sustainable alternative for the removal of xenobiotics from wastewater. The development of strategies combined with physical, chemical and biological methods is attractive for wastewater treatment. In the last decades several studies have reported hemolytic activity, not only in bacteria but also in fungi such as Aspergillus, Penicillium, Blastomyces, Coccidioides, Stachybotrys, Volvariella, Termitomyces, Rhodophyltus and Rhizopus. Additionally, fungal hemolysins such as Nigerlysin, Terrelysin Chrysolysin Stachylysin, Aegerolysin and Phallolysin have been well characterized. Although little is known about these fungal hemolysins, scientific interest for their application in different industrial sectors (including the biomedical and environmental field) is very high. The fungi extremophiles that have metabolic capabilities which allow them to grow in highly contaminated matrices and at the same time biotransform contaminants are of special interest. The characterization of wastewater generated in the meat industry such as slaughterhouses, have high indexes of organic load due to the presence of blood which is the main pollutant and generate contamination of about 200.000 mg/L BOD5. Moreover the wastewater also contains feces, stomach content, high levels of fat and also possesses fluctuations in the pH due to the presence of cleaning agents, high levels of nitrogen, phosphorus and salt, all this further complicating bioremediation. The aim of this study is: 1) To analyze the potential of the hemolytic activity of 10 strains of halophile fungi isolated in South Baja California, and to measure the hemolytic activity by the fungal strains of interest. 2) To explore the potential of these fungal strains in the bioremediation to try to solve the problem of water pollution generated in the Apatlaco river watershed, since it is considered an environmental heritage of the community of Morelos, because it is a major source of supplies for agriculture, economy and tourism in the state

Aldehyde dehydrogenase diversity in bacteria of *Pseudomonas* genus

Héctor Riveros-Rosas[1,2], Adriana Julián-Sánchez[1], Gabriel Moreno-Hagelsieb[2], Rosario Muñoz-Clares[3]

[1] Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México, CdMx, 04510, México. [2] Department of Biology, Wilfrid Laurier University, Waterloo, ON , N2L 3C5, Canada. [3] Departamento de Bioquímica, Facultad de Química, Universidad Nacional Autónoma de México, Ciudad de México, CdMx, 04510, México.
hriveros@unam.mx; Tel +52 (55) 5623 2284

Aldehyde dehydrogenases (ALDHs) comprise one of the most ancient protein superfamilies widely distributed in the three domains of life. Their members have been extensively studied in animals and plants, sorted in different ALDH protein families and its participation in a broad variety of metabolic pathways has been documented. Paradoxically, no systematic studies comprising ALDHs from bacteria have been performed in spite of their extensive metabolic capacity, their ability to produce multiple secondary metabolites, as well as their ability to use various carbon sources. Among bacteria, the genus *Pseudomonas* is one of the most complex bacterial genera and is currently the genus of Gram-negative bacteria with the largest number of species. *Pseudomonas* include a metabolically versatile group of organisms that are known to occupy numerous ecological niches. For this reason, we selected the *Pseudomonas* genus as a paradigm to analyze the diversity of ALDHs in bacteria. Thus, *Pseudomonas* strains with a complete annotated genome were retrieved from NCBI´s RefSeq genome database. The 258 selected *Pseudomonas* strains belong to 46 different species, along with 23 *Pseudomonas* with no species designation. The genomes of these *Pseudomonas* strains contain from 3315 to 6825 protein coding annotated genes. A total of 6510 protein ALDH sequences were found in the selected *Pseudomonas* strains, with a median of 24 ALDH genes per strain (by comparison humans possess only 19 different *aldh* genes). With no exceptions, all *Pseudomonas* strains possess several copies of *aldh* genes. *Pseudomonas saudiphocaensis* possesses the lowest number of *aldh* genes (9), but also possesses the smallest proteome (3315 protein coding genes). In contrast, *Pseudomonas pseudoalcaligenes* KF707 NBRC110670 possesses the maximum number of different *aldh* genes (49), with a proteome below the average size (5571 protein coding genes). All ALDHs found in *Pseudomonas* can be sorted in 43 different protein families, although 76% of all found ALDHs belong to only 14 different protein families. Here, it is interesting to note that many *Pseudomonas* species possess multiple copies of *aldh* genes that belong to the same family. ALDH families with the highest number of genes found in *Pseudomonas* are ALDH6, ALDH27, ALDH5, ALDH14, and can be considered as part of the core genome from *Pseudomonas*.

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Novel lncRNAs are induced as part of the neighbor proximity response in *A. thaliana*

Irving J. García López and Selene L. Fernández-Valverde
Unidad de Genómica Avanzada-Langebio, CINVESTAV
Libramiento Norte Carretera Leon Km 9.6, S/N, 36821 Irapuato, Guanajuato, Mexico.
Phone: +52 462 166 3034
Email: selene.fernandez@cinvestav.mx

Long non-coding RNAs (lncRNAs) are RNA molecules >200 nt long with very low coding potential that have been shown to be widespread across all kingdoms of life. Many lncRNAs have now been demonstrated to play gene regulatory roles, including participating in development and the establishment of chromatin structure in animals. However, only a dozen of lncRNAs have been functionally characterized in plants. In this work, we focused on the identification and annotation of lncRNAs that display differential expression in the response to neighbor proximity phenomenon induced by light source competition in *A. thaliana* seedlings. We assembled all transcripts across four timepoints post shade treatment (low-red light) in cotyledons and hypocotyls and identified 2,388 lncRNAs, including 159 that were not previously annotated in public databases. Of these, 83 were significantly differentially expressed in cotyledons and 514 in hypocotyls in response to shade treatment. To infer potential lncRNA functions, we functionally annotated 29 groups of genes tightly co-expressed with our lncRNAs, identifying functions related to hormone response and cell wall modification. With this work, we have expanded the list of known lncRNAs as well as identify candidate lncRNAs which may participate in the regulation of the response to neighbor proximity in plants.

Molecular and structural modeling of HPV-16 E6 oncoprotein and variants in the interaction with P53 as target protein

Hugo Alberto Rodríguez Ruiz1, Olga Lilia Garibay Cerdenares1,2, Berenice Illades Aguiar1, Maricela Sarita Montaño3 and Marco Antonio Leyva Vázquez1*
1Molecular Biomedicine Laboratory. Faculty of Chemical-Biological Sciences. Autonomous University of Guerrero, Mexico; hugordzrz@uagro.com; billades@uagro.com
2CONACyT- Autonomous University of Guerrero, Mexico; olgaribayce@conacyt.mx
3Faculty of Chemical-Biological Sciences. Autonomous University of Sinaloa, Mexico; montanovmar@gmail.com
*Correspondence: leyvamarco@yahoo.com; Tel.: +52 (747) 4710901

Abstract: Intratypical variants in the nucleotide sequence of LCR, L1 and E6 genes HPV-16 have been described. Amino acid changes of viral proteins such as E6 oncoprotein are involved in the differential cancer progression behavior, due to the modifications on the interaction networks generated by the E6 and their targets, mainly p53. Intratypical variants of E6: E-G350, E-A176/G350, E-C188/G350, AAa and AAc are the most frequent and related with differential oncogenic risk in Cervical Cancer in the South of Mexico.

Since the resolution of the 3D structure of the HPV-16 E6 oncoprotein it has been analyzed the interaction effect with some of its protein and drug targets. However, the effect of the intratypical variants in the 3D structure or the interaction with targets has not been evaluated.

Through an *in silico* approach, including molecular modeling, docking and molecular dynamics, it was generated the 3D structure of these variants, even though there are amino acid changes in the intratypical variants, the general 3D structure remains without change, but physicochemical properties did, such as isoelectric point and stability index. Also, it was analyzed the effect of the amino acid change in the E6-E6AP-P53 interaction. The results shown differential interaction profiles between reference and variants in P53-E6-E6AP, but no with direct E6-E6AP interaction, those different profiles were related with an increase of number and type of non-bonded interactions, mainly in AA variants in comparison with European variants (table 1). These results may explain the most aggressive behavior of Asiatic-American variants in cancer progression.

Table 1. *In silico* prediction of type and number of interactions between HPV E6 oncoprotein and intratypical variants.

Number:	Residue interactions	Salt bridges	Hydrogen bonds	Non-bonded contacts	Residue interactions	Salt bridges	Hydrogen bonds	Non-bonded contacts
HPV intratypical variant	*Peptide E6AP*				*Protein p53*			
E6 HPV16 Reference	16/9	5	10	87	18/23	5	18	174
E6 HPV16 E-G350	16/9	5	10	87	18/23	5	18	174
E6 HPV16 E-A176/G-350	16/9	5	10	87	18/23	5	18	174
E6 HPV16 E-C188/G-350	16/9	5	10	87	18/23	5	18	174
E6 HPV16 AAa	16/9	4	9	92	19/24	5	21	204
E6 HPV16 AAc	16/9	4	9	91	19/23	3	21	202

Genomic analyses on phytopathogenic nematodes

Luis Ángel Xoca-Orozco[1], Jonathan Alejandro Rodríguez-Martínez[1], Miguel Ramírez-Tixta[1], Julio César Amezcua-Romero[1], Leonel Rosas-Hernández[2], Julio Vega-Arreguín[1] Cristian Nava-Díaz[2] & Alejandra Rougon-Cardoso[1*]

*corresponding author: arougon@enes.unam.mx Tel. 01 (477) 194 08 00 ext. 43485
[1] Laboratorio en Ciencias Agrogenómicas, Universidad Nacional Autónoma de México (UNAM) ENES Unidad León, 37684, León Guanajuato, México.
[2] Instituto de Fitosanidad, Colegio de Posgraduados, Km. 36.5 Carretera México-Texcoco, Montecillo, Texcoco, Estado de México, CP. 56230, México.

Plant parasites are responsible of about 35% of crop losses worldwide and the loss of almost half of the production in developing countries. Parasites introduce a great amount of toxins and effector proteins into their hosts. If these effectors are detected by the host plant it may activate its immune system to defend itself. However, some parasites have evolved effectors that are capable of evading, modulating or even reprograming the plant immune system to reach colonization. Genomic research of parasitism and parasite-host interactions can provide a relatively fast understanding of the parasitic and resistance proceses, thus allowing the development of new biotechnological strategies to reduce crop losses. Our goals are to generate genomic data of parasitic nematodes, to understand their way of action at the molecular level, and to analyze how they have evolved in order to develop better and more environmentaly friendly strategies to avoid parasite infections.

We have generated genomic data of a phytopathogenic nematode which is hosted by more than 400 plants, including crops and weeds. This wide host range makes it difficult to erradicate. We will show the results of bioinformatics and comparative genomics analyses among various strains of this parasite and among other parasitic nematodes, focussing on the putative effector repertoire.

Diversity of metabolic profiles in BP8, an enriched microbial community capable of growing in a polyether polyurethane-acrylic varnish, unveiled by metagenomic analysis

[1]Ayixón Sánchez Reyes, [2]Ivan Liachko, [2]Shawn Sullivan, and [1]Herminia Loza-Tavera
[1]Departamento de Bioquímica, Facultad de Química, UNAM, Ciudad de México. 04510. MÉXICO.
[2]Phasegenomics, 4000 Mason Road, Fluke Hall / Suite 225, Seattle, WA 98195
USA. Tel. 52 (55) 5622-5280 e-mail: hlozat@unam.mx

Key words: Metagenomic deconvolution, polyurethane biodegradation, functional annotation

In this work, the genetic and functional potential of the BP8 enriched microbial community, capable to attack a polyether polyurethane-acrylic varnish (PolyLack) were investigated, by a metagenomic analysis using a Hi-C based method, a novel technique that allows to regenerate contiguity signals between sequences of the same genome. BP8 was selected from a landfill sample and cultivated for several generations in a mineral medium with PolyLack® as the sole carbon source. The varnish, besides the polymer, contains solvents such as N-methylpyrrolidone, isopropanol, butoxyethanol and glycol ethers. The deconvolution analysis suggested the presence of five distinct clusters with genome completeness from 89 to 97.7% that closely match to: *Paracoccus*, *Chryseobacterium*, *Parapedobacter*, a member of the Microbacteriace family, and *Ochrobactrum intermedium*. Three of these genomes seem to be totally new. Metagenomic functional analysis revealed that aromatic compounds metabolism had the highest abundance of sequences, suggesting a community common role in aromatics biodegradation. Novel reconstructed pathways for isopropanol, 2-butoxyethanol, dipropylene glycol (methyl and butyl) ether, based in the presence of key enzymes encoded in the individual genomes, suggest that these compounds could be targets of terminal oxidizations mediated by dehydrogenase activities, or can be attacked in the internal ether bonds by oxidoreductases. Besides, nine esterases and four novel amidases showed significant similarity to sequences that degrade polyurethane varnish Impranil and other type of carbamate compounds. These findings suggest that the BP8 community, besides of being able to attack polyurethane, can play important roles in the degradation of other environmental contaminants.
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Prediction of the 3D structure of *Aedes aegypti* acetylcholinesterase

Erika Amparo Torres Reyes, Víctor Mario Jaramillo Pérez, Mario Alberto Barón Rodríguez, Leonor Yamile Vargas Méndez*
Grupo de Investigaciones Ambientales para el Desarrollo Sostenible.
Universidad Santo Tomás. Carrera 18 # 9-27 Bucaramanga, Santander, Colombia.
(+57) (7) 6800801. leyavar@gmail.com

Abstract:

The *Aedes aegypti* mosquito is the principal vector responsible for the spread of viral diseases, such as Dengue, Zika and Chikungunya. The World Health Organization promote the vector control, as the first and best approach, to reduce and prevent those viral transmissions. To achieve this goal, insecticides are used; some of them are acetylcholinesterase (AChE) inhibitors. The development of new insecticides without resistant pest populations, by rational and biorational design, required the structure of the target; in this case the *Aedes aegypti* AChE (aaAChE).

As the aaAChE tertiary structure has not been resolved, our group propose to predict that structure by threading of sequence fragments (code Q6A2E2) with fragments of secondary structures proteins housed in the Protein Data Bank. Then, we run several molecular dynamics simulations (MD) to evaluate the stability and fragments flexibility of the models predicted.

Our results show the top five possible enzyme models predicted which corresponds to the five largest structure clusters. Every model has a globular structure with a catalytic triad (Glu325, Ser326 and His566) and hydrophobic residues (Trp211, Phe415 and Phe456); domains most commonly found in hydrolase. Furthermore, the top first model is structurally closed to the crystal structure of AChE catalytic subunit of the Malaria vector *Anopheles Gambiae*; specie in the same order (Diptera) as *Aedes aegypti*. The MD simulations show the stability of the predicted models and flexibility of fragments that cover the catalytic gorge; known as the lid.

In conclusion, the structural and dynamic results are promising to use it in the rational design of insecticide that promote the blockade of cholinergic neurotransmission in *Aedes aegypti*

DOES SPC13, AN ANTIMICROBIAL ACTIVITY FOUND IN THE VENOM OF THE *SCOLOPENDRA POLYMORPHA,* ALSO HAVE HISTONE H3 PROPERTIES?

[1]Carolina Abarca Camacho, [1]Eduardo Rivera Morales, [1]Lucero Valladares Cisneros, [1]Ma. Del Carmen Gutiérrez Villafuerte. Universidad Autónoma del Estado de Morelos.
[1]Centro de Investigación en Biotecnología. Av. Universidad No. 1001. Col Chamilpa. Cuernavaca, Morelos. C. P. 62209. (777)3297057 Ext. 3522, email: carmengu@uaem.mx.

Antimicrobial peptides are crucial elements of innate immunity for the protection of organisms against pathogens, these peptides have been found in plants, animals and other organisms.

On the other hand, histones with antimicrobial properties have been also proposed as components of the innate defense system, acting against pathogenic microorganisms in vertebrates and invertebrates (1, 2).

Recently, our group reported an antimicrobial peptide (SPC13), extracted from the venom of *Scolopendra polymorpha*, with a molecular weight of 13 kDa (3). Its partial sequence showed a sequence identity of 90% with respect to histone H3 of *Scolopendra viridis* (GenkBank: DQ222181.1). Therefore, in the present study it will be determined if SPC13 has characteristics of a functional histone.

The extraction of the venom from *S. polymorpha*, was performed by mechanical stimulation of the forciples. Thereafter, SPC13 peptide was purified by 16% SDS-PAGE and electroelution and its antimicrobial activity was assayed against *S. aureus* by the agar diffusion method. DNA and histones were obtained from the whole body of *S. polymorpha* according to Pecina (4) and Sidoli (5) respectively. SPC13 and histones recognition assay was performed by western blot with anti-histone H3; subsequently the sequences of histone H3 were aligned with the partial sequences of SPC13. Finally, a DNA-Protein binding assay was performed on a 1% agarose gel in which the histone binding was incubated with either DNA or SPC13 peptide to observe its interaction. Results showed that, although anti-histone H3 antibody did not recognize at SPC13, the DNA-Protein binding test demonstrated that SPC13 can interact with DNA.

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"Purification and characterization of the paralogous enzymes Bat1 and Bat2 of Saccharomyces cerevisiae"

Beatriz Aguirre-López, Eréndira Rojas, Marietta Tuena and Alicia González
Department of Biochemistry and Structural Biology, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México. P.O. 04510. México. Tel. (+52) 55 56225631; email: baguirre@ifc.unam.mx

Approximately 100 millions of years ago the lineages of *Kluyveromyces* and *Saccharomyces* diverged one from another and it is known that *S. cerevisiae* lineage was formed due to a hybridization event occurred after the *Kluyveromyces* appearance. The formation of a hybrid between the KLE clade and the ZT clade gave rise to the *S. cerevisiae* ancestor. In the *Saccharomyces* lineage various groups of duplicated genes were generated, sharing homology and synteny. Some of these paralogous genes are involved in nitrogen metabolism. *Sc*Bat1 and *Sc*Bat2 are two aminotransferases which arose from this polyploidization event, which participate in valine, leucine and isoleucine (VIL) metabolism. Previous results from our laboratory have shown that *ScBAT1* and *ScBAT2* display opposed regulatory profiles when the yeast is grown on either primary or secondary nitrogen sources, *ScBAT1* is expressed in presence of glutamine (Q) and repressed in VIL, conversely *ScBAT2* expression profile is induced in VIL and repressed in Q. *Sc*Bat1 and *Sc*Bat2 share 77% of identity. *Sc*Bat1 is located in the mitochondrion and *Sc*Bat2 is a cytoplasmic enzyme. In the present work we carried out the purification and determination of the biochemical and kinetical parameters of *Sc*Bat1 and *Sc*Bat2 in order to better understand whether functional diversification of these proteins was also dependant on modification of their kinetic parameters.

We used molecular exclusion chromatography, native gels and Light Scattering to determine their oligomeric conformation which is dimeric for the two enzymes. Using intrinsic fluorescence we observed that their denaturalization pattern is very similar among the enzymes: once denaturalized these enzymes can be 90% renatured, indicating that they have high stability. We also performed biochemical assays in order to determine their kinetic parameters (Km and Vmax) using the three substrates: valine, isoleucine and leucine and varying its substrate glutamic acid. It can be concluded that these isozymes have not diverged in their kinetic properties and oligomeric structure, thus, functional divergence was established through the acquisition of differential expression patterns and subcellular localization.

The role of renal betaine aldehyde dehydrogenase in the carnitine biosynthesis

José Alberto Alcantar-Ibarra[1], Melissa María Miranda-Cruz[1], Elisa Miriam Valenzuela-Soto[2], José Guadalupe Soñanez-Organis[1], Jesús Alfredo Rosas Rodríguez[1]*

Depto. de Ciencias Químico-Biológicas y Agropecuarias, Universidad de Sonora Unidad Regional Sur, , Navojoa, Sonora, México. [2]Centro de Investigación en Alimentación y Desarrollo (CIAD), Carr.a la Victoria KM. 0.6, Hermosillo, Sonora C.P. 83304, México.

*Corresponding author. Email. jrosas@navojoa.uson.mx

The enzyme betaine aldehyde dehydrogenase from mammals (BADH EC 1.2.1.8) belongs to the ALDH9 family involved in amino aldehyde detoxification; hence the specificity of the aldehyde substrate defines their role in polyamine catabolism, γ-aminobutyrate synthesis, carnitine, and glycine betaine biosynthesis. The renal betaine aldehyde dehydrogenase (pkBADH), is a cytosolic enzyme that has been biochemical and kinetical characterized as a highly specific enzyme to the betaine aldehyde substrate with Km_{BA} = 145.5 μM. Therefore, the pkBADH activity investigations are related to the glycine betaine accumulation to counteract the stress condition in the renal medulla. In contrast, the human and rat liver enzymes present better affinity parameters respect γ-trimethylaminobutyraldehyde (γ-TMABA) with $Km_{γ\text{-}TMABA}$ = 1.4 and 4.8 μM respectively, suggesting that these enzymes are implicated mainly in the carnitine biosynthesis. L-carnitine has a role in the β-oxidation and transport of long-chain fatty acids for energy generation. Recently investigations describe the kidney participation in the synthesis, turnover, secretion, and re-absorption of L-carnitine, and its implication to the reduction of the renal hypertrophy and renal tissue damage. In this work, we overexpressed the pkBADH enzyme, synthesize the γ-TMABA substrate, and evaluate the activity and initial velocity patterns for γ-TMABA. Our results demonstrate that pkBADH increase the activity in 1.5-fold for γ-TMABA in contrast to betaine aldehyde substrate, suggesting that pkBADH is involved in the carnitine metabolism in the kidney.

Development of a computational algorithm for designing inhibitors under a fragment-based approach

María Fernanda Álvarez Esquinca, Marcelino Arciniega Castro
Departamento de Bioquímica y Biología Estrucural, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México. Cto. Exterior s/n, Ciudad Universitaria, 04510 Ciudad de México, CDMX
Correo electrónico: marciniega@ifc.unam.mx Tel.: +52 55 562 257 03

This project seeks to establish a method that allows the design of inhibitors under a fragment approach. This approach takes care of the identification of small molecules (MW<250Da) called fragments, that interact with specific regions of the protein structure (Joseph-McCarthy, Campbell, Kern, & Moustakas, 2014). The integration of several of these molecules in one single molecule allows the development of a highly specific inhibitor (Doak, Norton, & Scanlon, 2016). Nevertheless, the identification of fragments along with their subsequent integration in one single molecule, are pretty complex tasks (Joseph-McCarthy et al., 2014). This project pursues, in first instance, to provide a reference study that evaluates molecular docking as a way to correctly pose fragments (Schulz, Landström, Bright, & Hubbard, 2011). A second stage will focus on the establishment of a Bayesian Belief Network (BBN) based on molecular interaction fingerprints of the ligands (Abdo, Beining, Christopher, Salim, & Willett, 2010) (Marcou & Rognan, 2007). In the third stage of this project, the aim will be to propose lead molecules starting from two or more fragments docked into the receptor using the program AutoClick Chem.

RNA Aptamer selection to *Rickettsia rickettsia*

Febe Jezarel Morales Valdez[a], Ana Mercedes Andrade Aguilar[a], Nicolas Addiel Serafín Higuera[b], Javier Gonzalez Ramirez[b], Rene Machado Contreras[a], Rafael Martinez Miranda[a], Julia Dolores Estrada[a], Ana Gabriela Leija Montoya[a,b].

[a]. Facultad de Medicina Mexicali, Universidad Autónoma de Baja California.
[b]. Unidad de Ciencias de la Salud, Universidad Autónoma de Baja California.

Rocky Mountain spotted fever (RMSF) is a tick-borne disease caused by Rickettsia rickettsii (R. rickettsii), the case fatality rate from outbreaks of RMSF in Mexico ranged from approximately 30% to 80%, some patients who recover from severe RMSF present long-term or permanent disabilities or amputations. An accurate and early diagnosis is important to initiate a specific treatment, but the diagnosis is challenging due to in the early stages of illness the symptoms are non-specific and similar to many other common infectious diseases [1].
Aptamers are oligonucleotide ligands, which are capable of adopt three-dimensional structures to bind tightly and specifically to their targets, from small inorganic molecules to proteins and even whole cells, with many potential uses including as a diagnostic tools. Aptamers have been compared to antibodies due to their high affinity and specificity. Moreover, aptamers are easier and more economical to synthesize than antibodies and can be modified to be used in biofluids [2].
The aim of this study is to isolate RNAs aptamers for *R. rickettsii* antigens from a combinatorial library of 4_{15} variants using a modified SELEX method (Systematic Evolution of Ligands by EXponential enrichment). The RNA aptamers were obtained from 3 pre-selection cycles against whole blood, 3 pre-selection cycles against *R. parkerii* and 5 selection cycles against *R. rickettsii*. The *in silico* secondary structure analysis shows a 6 base stem and rich CU loop pattern, possible involved on binding. Individual affinity aptamer determinations are in process.

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The regulation of metabolic syndrome and hepatic damage in an experimental model by an adenosine derivated.

Alejandro Rusbel Aparicio-Cadena, María Guadalupe Lozano-Rosas, Enrique Chávez, Gabriela Velasco-Loyden and Victoria Chagoya de Sánchez. Instituto de Fisiología Celular, UNAM, Del. Coyoacán, 04510, Cd. Mx. México. Tel: +52(55)5622-5614. Fax: 56225614. E-mail: vchagioya@ifc.unam.mx

The metabolic syndrome is a multifactorial condition originally associated with cardiovascular diseases and whose main trigger is usually obesity or overweight, referred to excessive consumption of fats and sugars. The consequent high energy content can alter the hepatic metabolism.

Non-alcoholic fatty liver disease is a condition that is most often established in people with metabolic syndrome.

It has been described that liver damage in people with metabolic syndrome is consistent with two events: The first one is due to insulin resistance directly linked to overweight or obesity. In this phase the liver presents steatosis. The second, generated by excess lipids accumulated within the hepatocytes, which leads to mitochondrial dysfunction, with the consequent generation of oxidative stress and inflammation (steatohepatitis).

On the other hand, adenosine is a nucleoside involved considerably in the intracellular energy production and whose pharmacological use has been shown to have a hepatoprotective effect in experimental models of cirrhosis and partial hepatotectomy.

In this work, an experimental model was standardized where from a high-calorie diet (High-Fat Diet), which administered daily for six weeks in rats of the Sprague-Dawley strain, established the clinical spectrum of metabolic syndrome, as well as liver damage: Overweight, hyperinsulinemia, hyperglycemia, hyperlipidemia and steatohepatitis.

In another erxperimental group, simultaneously to said diet, an adenosine derivative -IFC-305- was administered intraperitoneally. At the end of the treatments, this group showed improvement in liver histology, as well as liver lipoperoxidation levels, mitochondrial function and serum indicators of metabolic syndrome: insulin and colesterol concentration. These results suggest that the IFC-305 compound could maintain the energetic metabolism, attenuating the damage produced by a hypercaloric diet.

The control group, treated with physiological saline, showed the basal levels of the mentioned parameters.

It will seek understanding the molecular mechanisms by which these effects are occurring.

Validation of biomarkers for the detection of *Helicobacter pylori*-associated gastric cancer.

Arenas Linares Ana Silvia1, 2, Eva Hernández Márquez2, Diana Lashidua Fernández Coto2, Elba Cristina Villegas Villareal1*, Guadalupe Ayala Aguilar2*.
1Universidad Autónoma del Estado de Morelos, 2Instituto Nacional de Salud Pública. Av. Universidad 655 CP. 62100. Cuernavaca Morelos. Tel. 7773293000 ext 2231. *Correspondence should be addressed to Guadalupe Ayala Aguilar; gayala@insp.mx or Elba Cristina Villegas Villareal; elbav@uaem.mx.

The methods currently available for the detection and the prognostic evaluation of gastric cancer (GC) have insufficient sensitivity and specificity, which is why they are not recommended as diagnostic tests. The identification of biomarkers is necessary for the early detection of GC. Using comparative proteomics based on the 2D-DIGE strategy, ten plasma samples were analyzed. Five of them corresponded to cases of intestinal GC, and five corresponded to patients with non-atrophic gastritis (NAG). The generated 2D-DIGE images were statistically analyzed using the DeCyder™ 2D software. Twelve proteins were detected, and they showed a differential expression, by a factor of two or more times, between both groups. Seven of these proteins increased their expression, and five decreased it in the GC group, compared to the control group. The next step consisted in the separation of the plasma proteins into two-dimensional preparative gels. The selected spots were manually cut and identified by MALDI-TOF-MS. The MS/MS spectra obtained were analyzed by means of comparative bioinformatics methods with the Protein Pilot software using a database from the UniProt page (http://www.uniprot.org). The results were validated by a double search in Mascot and in Xtandem. Subsequently, based on background literature on the proteins that are altered during the GC process, three proteins were selected: Vitamin D-binding protein (VTDB), Clusterin (CLUS), and Inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4). These proteins were validated by means of the western blot test, using the plasma of patients diagnosed with NAG and GC. The differences in the expression levels of each of these proteins in the plasma samples were determined by means of the U Mann-Whitney test. We found that the expression of CLUS was significantly lower in the GC group ($p = 0.010$). The expression of VTDB was found to be in lower concentrations among patients with GC, although it was marginally significant ($p < 0.068$). In the case of ITIH4, the validation by western blot showed that its concentration was higher among patients with GC; however, the difference was not significant. In conclusion, the Clusterin protein could be considered as a possible biomarker for the diagnosis of *Helicobacter pylori*-associated gastric cancer.

Characterization of the reaction center spectrum of *Rhodovibrio salinarum*.

Andrés Arenas Navarro, Claudia Peña Segura and Heliodoro Celís Sandoval.
Department of Molecular Genetics, Institute of Cellular Physiology. UNAM
University City. 04510. Mexico City, Tel. 015556225667 aarenasna@gmail.com

Rhodovibrio salinarum is a photosynthetic bacterium that belongs to alpha proteobacteria, is gram-negative, spiral-shaped, 0.6 to 0.9 microns wide, mobile through polar flagella and reproduced by binary fission. The photosynthesis is carried out in the invaginations of its internal membrane in the form of a vesicle, where the reaction center contains alpha-bacteriochlorophyll and several carotenoids, it also contains ubiquinones Q-10 and menaquinones MK-10 as main carriers of electrons (Imhoff *et al*, 1998).
According to the characterization of its growth carried out in different concentrations of NaCl, the optimum development of this halophilic bacteria is in concentrations between 16% and 22% (2.73M to 3.76M). However, they can grow in concentrations of 3% to 25% (0.51M to 4.27M).
It was observed that the spectra of the photosynthetic membranes in these conditions modify their absorption depending on the salinity of the medium.
In this work *Rhv. salinarum* was exposed to 18% concentration of NaCl and we observed that in the chromatophores of the membranes at the moment of oxidation a change in the absorbance occurred. In addition, the reaction centers were isolated according to Jolchine *et al*. and Codgell *et al*. Where the fraction of the reaction centers was found to the antenna complexes present. When this fraction is oxidized with potassium ferricyanide (K4Fe (CN) 6), a loss of absorbance was found at 796 and 847 nm, similar to that found in chromatophores (Duysens, 1957).
We postulate that the reaction centers are attached to the antenna complex, hoping to verify it with gradients of sucrose and electrophoresis with and without detergent, also comparing them with the reaction centers of *R. sphaeroides*.

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CONSTRUCTION OF AN ENDOXIFEN BINDING PROTEIN WITH HIGH AFFINITY AND SELECTIVITY

Emma Liliana Arévalo-Salina, Humberto Flores and Gloria Saab-Rincón
Departamento de Ingeniería Celular y Biocatálisis, Instituto de Biotecnología, Universidad Nacional Autónoma de México. Av. Universidad 2001 Col. Chamilpa 62210 Cuernavaca, Morelos, México.
(777) 3292605 emmaar@ibt.unam.mx

Tamoxifen is a prodrug used in hormone therapy against breast cancer that is transformed by the liver in 4-hydroxy-N-demethyltamoxyphene (endoxifen), the real active principle, and 4-hydroxy tamoxifen (4OHT). The response to treatment shows a great variation, mainly due to the variation in the final concentration of endoxifen. Besides, the therapeutic range is limited due to its toxicity. In order to maintain a therapeutic range of this metabolite, it has been proposed to monitor the concentrations through the use of biosensors. These devices use a biological element for recognition of a target molecule, which is a fundamental part in the development of biosensors. In our group we are interested in obtaining a high affinity and selectivity protein to bind endoxifen that will serve as the foundation for the construction of an endoxifeno biosensor to monitor its concentration in blood, making possible an individualized therapy with this drug.

We decided to use the human estrogen receptor alpha (ERα) as the recognition element. This molecule has already a high affinity for endoxifen, but has a high level of promiscuity towards a large variety of compounds. The latter limits its use as a possible recognition molecule in the development of biosensors. Because of this, we seek to redesign the ligand binding domain (LBD) of human ERα in a way that preserves its high affinity to endoxifen, while decreases considerably its affinity towards natural estrogens, especially towards E2. To achieve this, the rational modification of specific contacts mediated by hydrogen bonds in the E2 biding was proposed, as well as the optimization of surrounding amino acid residues to generate protein-ligand interactions specific for endoxifen. To predict these changes, a computational redesign was proposed, taking into account the structural information that has been previously reported for the interactions of the LBD of ERα with estradiol and metabolites of tamoxifen. Molecular modeling can predict substitutions of amino acid residues that could be beneficial. Algorithms such as those used by Rosetta calculate free energies based on steric collisions, hydrophobic packing, hydrogen bonds and electrostatic interactions.

Modulating hydrolysis/transfer function through the analysis of inter-residue protein contacts in the alpha-amylase enzyme family

Rodrigo A. Arreola-Barroso, Wendy Xolalpa-Villanueva, Leticia Olvera-Rodríguez, Alfonso Miranda-Molina, Gloria Saab-Rincón.
Instituto de Biotecnología, UNAM
Av. Universidad #2001, Col. Chamilpa C.P. 62210
+52 (777) 329-1640
gsaab@ibt.unam.mx

Sugar moieties mediate molecular recognition, modify drugs biological activity and pharmacokinetics, but their study has been impaired by its synthesis. Synthetic approaches require the discrimination of sugar hidroxyl groups to attain the right product. Traditional chemistry achieves this with protecting groups, which must be removed after the desired chemical step is performed. Nature usually adds sugar moieties previously activated with nucleotides, which is expensive to implement at industrial level. Oligo- and polysaccharides, like starch or sucrose, are abundant sources of activated sugars. Glycanotransferases transfer sugar moieties to other hydroxyl groups and can thus become tools for glycosynthesis. Despite its synthetic potential, glycanotransferases are available only in those cases where a hydrolase has evolved into a transferase. Understanding the differences between glycosyl hydrolases and glycanotransferases might help creating predictive tools to favor transfer reactions by enzymes where evolution has only selected the hydrolysis reaction.

This work is an attempt to use the information contained in the inter-residue protein contacts to identify elements modulating and controlling the hydrolyisis and transfer functions of the alpha-amylase family. For that purpose we compared 3D structures of the hydrolases and transferases within the alpha-amylase family through the analysis of their inter-residues protein contacts. We identified that the inter-residue protein contact pattern is a signature that differentiates enzymes capability of transferring glucose units preferentially to water from those with an ability to transfer them to other molecules. We then attempted to exchange functions between a hydrolase and a transferase using site directed mutagenesis, obtaining variants with a modified hydrolysis/transfer ratio. In the case of the hydrolase we also obtained the kinetic parameters for the hydrolysis reaction of variants corroborating that mutations changed the hydrolyisis catalytic efficiency when compared to the wild type enzyme.

In conclusion, we were able to modify the hydrolysis/transfer ratio through designed mutagenesis.

Phaseolus vulgaris rhizosphere stimulates *Rhizobium* conjugation: home, food and love?

Luis Alfredo Bañuelos-Vázquez(1), Gonzalo Torres Tejerizo(2), Lourdes Girard(3), Laura Cervantes de la Luz(1), David Romero(1) and Susana Brom(1).

Programas de Ingeniería Genómica(1) and Biología de Sistemas y Sintética(3), Centro de Ciecias Genómicas, UNAM. Av. Universidad 1001, col. Chamilpa, Cuernavaca, Mor., México. alfred@ccg.unam.mx. IBBM CCT-CONICET-La Plata, Universidad Nacional de La Plata. La Plata, Argentina(2).

Rhizobium etli CFN42 is a Gram-negative bacterium, of great agricultural importance due to its symbiotic association with *Phaseolus vulgaris*, through the formation of nodules. The genome of *R. etli* CFN42 consists of one chromosome and six large plasmids. Among these, pRet42a has been identified as a conjugative plasmid, transferred at a high frequency (10-2) in laboratory conditions. The expression of the transfer genes is regulated by a "*quorum sensing*" system that includes a *traI* gene which encodes an homoserin lactone (HSL) and two transcriptional regulators (TraR and CinR). The objective of this work is to determine the role of plant-related compounds on the conjugative transfer (CT) of pRet42a. In a parallel work we have shown that pRet42a can perform CT inside nodules (see abstract by Brom et al.,). Also, we observed an increase in the CT frequency in the plant rhizosphere. For that reason we decided to verify if this increase is due to some plant compound. First, we determined that total bean root exudates and extracts increase the frequency of conjugation. This may be due to the plant providing better growth conditions and/or a physical support for the bacteria. Also, we tested if some plant compounds could substitute the bacterial *traI* encoded HSL to activate the conjugation machinery. The results were negative for plant compunds, as well as for HSLs from other bacteria. Finally, we verified individual compounds of the plant exudates, among these, some increased and others decreased the CT. With these results, we suggest that the plant participates at different levels to affect the CT, and that some compounds could be activating genes in the conjugation machinery.

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Effect of glucose concentration on the activity of ubiquitin E3 ligase of Mdm2

Raul Barzalobre Geronimo, Clara Ortega Camarillo, Luis Arturo Baiza Gutman, Gisela Ceballos Cancino, Alejandra Contreras Ramos.

Unidad de Investigacion Medica en Bioquimica. Hospital de Especialidades Bernardo Sepulveda. Centro Medico Nacional Siglo XXI. Instituto Mexicano del Seguro Social.
Facultad de Medicina. Posgrado en Ciencias Biologicas. Universidad Nacional Autonoma de Mexico.
SMZ 1 MZ 54 Edificio 3B. Colonia Ejercito Constitucionalista. Delegacion Iztapalapa. Ciudad de Mexico. C.P 09220.

The degradation of p53 occurs via ubiquitin-26S proteosome, after Mdm2 (murine double minute 2) marks p53 with ubiquitins. The concentration and phosphorylation of Mdm2 and p53, greatly affect the ubiquitination rate of p53, by avoiding the recognition and physical interaction between p53 and Mdm2. Phosphorylation of Mdm2 in Ser395 by ATM decreases its ability to direct the degradation of p53. Therefore, we aim to study the effects of high glucose concentrations on the RING domain of Mdm2 and its ubiquitin E3 ligase activity.

The results show that the stress caused by high concentrations of glucose stimulates the phosphorylation of Mdm2 in Ser 395 by ATM activation. The phosphorylation of Ser395 alters the RING domain of Mdm2 and decreases its activity of ubiquitin E3 ligase, ubiquitination and degradation of p53. Therefore, stress due to increased glucose decreases the activity of ubiquitin E3 ligase of Mdm2 by inducing the phosphorylation of this residue, which leads to the stabilization and increase of p53 and the subsequent activation of apoptotic events.

The Ser 395 residue of the RING domain is important for the efficient function of ubiquitin ligase of Mdm2 in β cells under conditions of hyperglycemia.

Participation of residue E261 in the structural stability of the glucose-6-phosphate dehydrogenase from *Pseudomonas aeruginosa*

Edaena Benítez-Rangel y Roberto Velasco-García. Facultad de Estudios Superiores Iztacala, UNAM. C. P. 54090. Tel. 56 23 12 60.
robertvela2001@yahoo.com.mx

P. aeruginosa is the causative agent of frequent intrahospital infections. Its enzyme glucose-6-phosphate dehydrogenase (G6PDHPa) participates in the EDEMP cycle, transforming glucose-6-P (G6P) to 6-P-glucono-delta-lactone and simultaneously reducing the coenzyme NAD(P)+ to NAD(P)H. Several studies highlight the importance of this enzyme in the adaptation of the microorganism to the oxidative stress that is frequently found in the tissues that it invades. Considering this, and the difficulty in combating infections caused by *P. aeruginosa*, we are interested in studying its G6PDH to know if it could be considered a potential target of antipseudomonal compounds. As an initial objective, we decided to investigate some of the structural features of the protein, so we now know that the tetramer, formed by the interaction between two dimers, is the main active form of the enzyme. With the same purpose, in a recent study, we demonstrated that the binding of the G6P substrate causes a conformational change in G6PDHPa that results in a protein with a quaternary structure superior to tetramer. This protein also is thermally more stable, more resistant to trypsin proteolysis and with greater affinity to this substrate (see "Structural stability of the enzyme glucose-6-phosphate dehydrogenase of Pseudomonas aeruginosa ..." in this Abstract book). In the search for residues that could be involved in this stability or participate in the mentioned conformational changes, in the present study we analyze a structural model of G6PDHPa obtained from the crystal coordinates of the human enzyme deposited in the Protein Data Bank. Unlike the human enzyme, which has eight saline bridges on the interdimeric surface, G6PDHPa has only four, and in which the residuals Glu261 and Lys264 of each subunit participate. Then, and to know the degree of participation in these bonds, we designed and obtained, by site-directed mutagenesis, the E261D and E261Q mutants of the enzyme. Later, we overexpressed them in *E. coli* and purified them by a method that we usually use for the wild enzyme. The purified mutants, together with the wild-type enzyme, were then kinetically characterized and used to determine the dissociation constant (Kd) of each of the Enzyme-G6P complexes and the concentration of urea at which each enzyme is half-inactivated (*c*50). Kinetic characteristics and *c*50 were obtained with enzymes preincubated without and with 6 mM G6P. The general methodology involved monitoring the enzyme activity by NADPH spectrophotometry at a wavelength of 340 nm, and structural changes by fluorometry, following the emission of the ANS fluorescent probe between 400 and 600 nm. The main results show that, as for thermal inactivation and proteolysis, G6P stabilizes the enzyme and increases its resistance to urea denaturation. Also, the replacement of Glu261 with Asp produced an enzyme with higher affinity for G6P, and in the presence of this compound the mutant E261D was more resistant (*c*50 = 2.98 M) than the wild-type enzyme (*c*50 = 2.54 M). On the other hand, the replacement with an uncharged polar amino acid, Gln, in the E261Q mutant, produced a tetrameric enzyme highly sensitive to dilution and with a strong tendency to dissociate to dimers. These results are suggesting that E261 is important in the maintenance of the quaternary structure of the enzyme, participating in the formation of the salt bridges between dimers.

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Differential Gene Expression Analysis Between *Heliopsis longipes* and *Heliopsis annua* to Identify Genes Related to Alkamides Biosynthesis

Génesis V. Buitimea-Cantúa[1,2], and Jorge Molina-Torres[1]*
[1] Centro de Investigación y de Estudios Avanzados. Km 9.6 Libramiento Norte Carretera Irapuato-León C.p 36821 Irapuato, Guanajuato, México. Tel. (52)-462 6239600
[2] Tecnologico de Monterrey, Centro de Biotecnología-FEMSA, Escuela de Ingeniería y Ciencias, Campus Monterrey. Av. Eugenio Garza Sada 2501, Monterrey, N.L., C.P. 64849, México.
*jmolinat@ira.cinvestav.mx

ABSTRACT

Heliopsis longipes accumulates high levels of alkamides in roots, but not in aerial part. A close related species, *Heliopsis annua*, does not produce alkamides, making this genus a suitable model to the study of the biosynthesis of these compounds.
The present study generates transcriptome resource for *H. annua* and describes the differential gene expression analysis with *H. longipes.* To the comparative analysis, the profile leaf and root transcriptomes of *H. annua* by RNAseq were generated from the polyA-enriched cDNA libraries. The transcriptomes were sequenced by RNAseq using Illumina HiSeq 2500 platform. The short reads were filtered, processed,assembled and analyzed. Differential expression analysis by EdgeR showed a major variation among the tissues of *H. annua*, in comparison with *H. longipes* tissues. A total of 2,058 transcripts in *H. longipes* and 5,818 transcripts in *H. annua* were differentially expressed. *H. annua* transcriptome displayed several differences between roots and leaves tissues i.e. 4,997 transcripts were down-regulated and 821 were up-regulated. Difference of gene expression among *Heliopsis* species revealed, as expected, that expression patterns in *Heliopsis* vary depending on the tissue type and species. 1,399 down-regulated transcripts were detected in *H. longipes* roots and these include those related to photosynthesis, carbohydrate metabolism, and other processes. 659 transcripts are up-regulated. Among the upregulated transcripts, 56 are proteins of unknown function. A set of candidates genes related to alkamides biosynthesis were selected and analyzed by RT-qPCR. This analysis was performed comparing: roots vs. leaves of *H. longipes,* and *H. longipes* roots vs *H. annua* roots. Genes assayed by RT-qPCR present a preferential expression in *H. longipes* roots, the species and tissue where alkamides are produced. These results confirm that these selected genes are potential candidates to alkamides biosynthesis in *H. longipes* roots.

The amino-terminal region of Pet309 is important to interact with its mRNA target and with mitoribosome.

Yolanda Camacho-Villasana; Angelica Zamudio -Ochoa; Aldo E. García-Guerrero; Xochitl Pérez-Martínez.
Departamento de Genética Molecular, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México (UNAM). Circuito exterior s/n. Ciudad Universitaria. Coyoacán, 04510. Ciudad de México.

In yeast, mitochondrial translation initiation is not well understood. Unlike bacteria, the mitochondrial mRNAs do not contain a Shine- Delgarno sequence for the ribosome to identify the *AUG* start codon. Mitoribosomes do not seem to scan mRNA to find the initiation *AUG*. Instead, mitochondrial ribosomes identify the translation initiation start codon with the assistance of proteins named translational activators. These proteins interact with specific mitochondrial mRNAs and with the mitoribosome (1,2). In the present work we characterized the protein Pet309, one of the translational activators specific for the mitochondrial mRNA *COX1.* This mRNA codes for Cox1, the largest subunit and essential component of the catalytic core of Cytochrome *c* Oxidase (C*c*O).

Pet309 contains 23 pentatricopeptide repeats (PPR) along its structure (3), and we have demonstrated that are important for binding to the *COX1 mRNA (4)* and to mitochondrial ribosomes (Zamudio-Ochoa, manuscript in preparation).

We previously observed that a mutant lacking 12 central PPRs of Pet309 reduced interaction with the *COX1* mRNA (4). In order to identify which region of Pet309 is important for interaction with the mitoribosome and the *COX1* mRNA, we generated a mutant lacking the first 6 PPRs present in the amino terminal (*pet309Δ53-311*) and a mutant lacking the last 4 PPRs in the carboxyl-terminal end of Pet309 (*pet309Δ760-962*). These mutants were not able to grow in respiratory media. We observed that *pet309Δ760-962* mutant protein was associated with the ribosome, and with the *COX1* mRNA, while the *pet309Δ53-311* amino terminal mutant protein showed no association with the mitochondrial ribosome or the *COX1* mRNA.

We conclude that even though the C-terminal end of Pet309 is necessary for C*c*O function, association with the mitoribosome or the *COX1* mRNA was not affected. In contrast, the Pet309 amino terminal end is necessary for interaction of Pet309 with the *COX1* mRNA and with the mitoribosome.

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CHEMICAL AND BIOLOGICAL ANALYSIS OF METHANOLIC EXTRACT FROM POMEGRANATE (*Punica granatum* L) LEAVES WITH ANTIBACTERIAL ACTIVITY AGAINST *Ralstonia solanacearum*

Manuel Carrillo-Morales,* Diana Sofía Narváez-Martínez, Eduardo A. Patiño-Guerrero
Universidad Politécnica del Estado de Morelos (Upemor)
Boulevard Cuauhnáhuac No. 566, Col. Lomas del Texcal, Jiutepec, Morelos, México. C.P. 62550.
Tel.: 777 229 35 33, *mcarrillo@upemor.edu.mx

Some microorganisms have the capacity to cause severe diseases in plants of agricultural and economic importance, causing considerable losses in their production. In the case of tomato (*Solanum lycopersicum*) one of the bacterial pathogens that causes great damage is *Ralstonia solanacearum*, causal agent of bacterial wilt, and whose treatment based on pesticides has not been sufficient to prevent its proliferation, making it necessary to search for alternatives that contribute to its control. Several research groups have reported *in vitro* inhibitory activity of plant extracts against *R. solanacearum*. In some cases, the analytical evidence indicates that inhibition is caused by secondary metabolites with general chemical and structural characteristics; in the vast majority of reports, the results are limited to determine the inhibitory activity of plant extracts, excluding the chemical identification of the substances involved. Similarly, although there are considerable studies about the antimicrobial activity of characteristic species of the Mexican flora against bacteria of clinical interest, there are few reports concerning their activity against phytopathogenic bacteria. In this context, the objective of the present work is to study the inhibitory activity of pomegranate (*Punica granatum* L.) against *R. solanacearum*. The experimental work consisted in preparation of the organic extracts from the leaves of the plant by gradient of polarity using hexane, ethyl acetate and methanol. Subsequently, the antibacterial activity of the extracts against test microorganism was evaluated by disk and well-diffusion methods, then the active extracts were fractionated by column chromatography. From the active chromatographic fractions, the major compounds were isolated by successive chromatographies. Antibacterial activity of extracts and active fractions was determined by measuring inhibition zones or by evaluating the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). Likewise, from the methanolic extract it was possible to isolate, by successive chromatographies, four compounds whose purity, however, is not sufficient to elucidate its structure by nuclear magnetic resonance. Current efforts are focused on obtaining these compounds in sufficient quantity and purity for an adequate spectroscopic analysis.

GLP-1r Activation Decreases HIF-1A Expression in the Kidney after Ischemia Reperfusion in Obese Rats

Susana Del Carmen Castro-Meza[1], Max Thorwald[2], Ruben Rodriguez[2], Akira Nishiyama[3], José Guadalupe Soñanez-Organis[1], Rudy M. Ortiz[2]*.

[1]Universidad de Sonora, Depto. de Ciencias Químico-Biológicas y Agropecuarias, Universidad de Sonora Unidad Regional Sur, Apartado Postal 85880, Navojoa, Sonora, México.
[2]School of Natural Sciences, University of California Merced, 5200 N Lake Road, Merced, CA 95343, USA.
[3]Kagawa University, Department of Pharmacology. 1-1 Saiwaicho, Takamatsu, Kagawa Prefecture 760-0016, Japan.
*Corresponding author:
PhD. Rudy M. Ortiz
School of Natural Sciences
University of California Merced
5200 N Lake Road, Merced, CA 95343, USA.
Email: rortiz@ucmerced.edu

ABSTRACT:

Acute kidney injury (AKI), a global health problem that affects approximately 13 million people a year, represents one of the primary complications of renal injury due to ischemia-reperfusion. Ischemia-reperfusion occurs in many clinical scenarios, including renal transplantation, vascular surgery, and shock. Increased oxidant production and/or the decrease of antioxidant enzymes, contribute to the tissue damage that occurs during ischemia- reperfusion-reoxygenation injury (IRRI). Glucagon-Like peptide-1 receptor (GLP-1r) activation has renoprotective effects in diabetes through its anti-inflammatory action. However, it is not known whether GLP-1r activation improves the renal antioxidant response after ischemia-reperfusion. Therefore, the objective of this study was to assess whether the renal protective effects of GLP-1r activation on renal IRRI reduces the oxidative stress and the hypoxia. We assessed the gene expression of HIF-1 a, NOX4, HMOX1, SOD1 and SOD2 in the kidney before and after 30 minutes of ischemia and 24 hours of reperfusion in the following group of rats: (1) untreated Long-Evans Tokushima Otsuka rats (n=7), (2) untreated Otsuka Long-Evans Tokushima Fatty rats (OLETF) (n=8), and (3) OLETF+GLP-1r agonist (EXE; 10 µg exenatide/kg/day (n=6). The induction of ischemia-reperfusion increased HIF-1α mRNA expression in untreated OLETF approximately 4-fold compared to LETO group, and OLETF + GLP-1r decreased it 100% compared to LETO group and 6-fold compared to untreated OLETF. The preliminary data suggest that GLP-1r is protective against renal IRRI due to the decrease in the gene expression of HIF-1α during ischemia-reperfusion and during chronic treatment in insulin resistant rats.

Characterization of gluconeogenic activity in Intermittent Fasting

Silvia Cristina Cervantes Gutiérrez, Deysi Gasca Martínez, Mauricio Díaz Muñoz
Institute of Neurobiology, National Autonomous University of Mexico
Blvd Juriquilla 3001, Querétaro, 76230, México. Tel. 442 238 10 35 ext. 34035.
crcervantes274@gmail.com, gasca@inb.unam.mx, mdiaz@comunidad.unam.mx

During fasting, the liver is responsible for maintaining blood glucose levels by regulating metabolic pathways such as gluconeogenesis, glycogenolysis and glycogen synthesis.

Gluconeogenesis is the metabolic pathway that aims to form glucose *de novo*, from non-carbohydrate precursors, is carried out mainly in the liver and to a lesser extent, in kidney. Its regulation occurs due to the activity of two limiting enzymes: phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), which respond transcriptionally to the presence of hormones such as insulin, glucagon and glucocorticoids, in addition to the redox state (Petersen et al., 2017).

Intermittent fasting (IF) is a dietary protocol that consists of periods without access to food, alternating with periods with free access to food (*ad libitum*) (Chausse et al., 2015). The chronic regimen of the IF carries different responses to those observed in an acute fasting or continuous restriction (Secor and Carey, 2016).

We evaluated the gluconeogenic response in Wistar male rats, in the following experimental groups: *ad libitum*, acute fasting, refeeding (24 h of fasting, followed by 24 h of ad libitum), and intermittent fasting (24 h of fasting, followed by 24 h of free access to food, during 10 days). Through indirect calorimetry, the respiratory coefficient was determined in all groups. The respiratory coefficient was calculated with the relation of the volume of CO2 produced on the volume O2 consumed during the treatment, both measured with metabolic cages. These results suggest that there is a metabolic adaptation in the liver of rats in intermittent fasting that is associated with an optimization of non-continuous nutrient intake.

Experiments will be presented that will include: the measurement of the gluconeogenic response, from the perspective of enzymatic activity, using reactions that allow to quantify spectrophotometrically the rate of conversion of oxaloacetate to phosphoenolpyruvate by PEPCK, and the dephosphorylation of glucose-6-phosphate by the G6Pase. As well, the content of both enzymes in liver cell fractions by Western Blot. It is also planned to evaluate the transcriptional activity of the genes of both enzymes by RT-PCR and analyze the histological localization of the marker enzymes of the pathway.

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Ligand based pharmacophore modeling and virtual screening to find new benzimidazole derivatives as potential PTP1B inhibitors.

Alondra Sarahi Chaidez Avila, Claudia Isela Avitia Domínguez, María Alicia Hernández Campos, Marcelo Gómez-Palacio Gastelum, Jorge Cisneros Martínez, Alfredo Téllez Valencia.
Facultad de Medicina y Nutrición, Universidad Juárez del Estado de Durango, Av. Universidad s/n, Centro, C.P. 34000, Victoria de Durango, Durango, México.
Tel: 6188121687; atellez@ujed.mx

Abstract

Due to its complexity and the inability of the treatments to maintain their effectiveness for long periods, Type 2 diabetes is a disease of great clinical, social and economic importance worldwide. In the search for new drugs, the protein tyrosine phosphatase 1B (PTP1B) is considered as a good target because of its role as a negative regulator of insulin signaling. In a previous work, we reported some benzimidazole derivatives as PTP1B inhibitors. With the aim to explore a greater variety of this chemical scaffold, the benzimidazole dataset of Chembridge library was analyzed through a ligand based pharmacophore and virtual screening strategy. First, a pharmacophore model was constructed using LigandScout software and the PTP1B inhibitors reported with a Ki value under 5 μM. To continue, the chemical dataset of 9418 benzimidazole derivatives was filtered using the phamacophoric model. After this, the molecules that passed the filter were subjected to a virtual screening procedure using Glide, but additionally to the binding energy, their interactions with the sites A, B, C, and D, in the PTP1B structure was taken into account. This was important because selectivity against its closest homologous T-cell PTP (TCPTP) is a concern. At the end, the ten benzimidazole derivatives with the highest binding energy and the best interactions with the four sites were selected. To analyze their potential selectivity, their binding mode in TCPTP was studied. Additionally, their druglike properties were predicted with DataWarrior software. Results suggested that the new benzimidazole derivatives have the potential to inhibit PTP1B and to be selective in respect to TCPTP. Therefore these molecules serve as hits to develop a new drug against this disease.

EFFECT OF IFC-305 ON AUTOPHAGY IN THE SEQUENTIAL MODEL OF CIRRHOSIS-HCC AND IN VITRO.

Chávez E., Velasco-Loyden G., Lozano-Rosas MG., Aguilar B., Muciño-Hernández G, Castro-Obregón, S., and Chagoya de Sánchez V.
Instituto de Fisiología Celular , UNAM. Circuito Exterior s/n Coyoacán, Ciudad Universitaria, C.P. 04510. Ciudad de México.
Correo electrónico: vchagoya@correo.ifc.unam.mx

Autophagy is a cellular process that helps to maintain a positive energy balance through the cell components degradation. Autophagy is known as mitophagy when it degrades mitochondria, a specialized mechanism of malfunctioning mitochondria elimination. Besides, it has been suggested an important role of autophagy for the suppression of spontaneous tumorigenesis. Previously, our group have been demonstrated the IFC-305 chemopreventive effect in the sequential model of cirrhosis-hepatocellular carcinoma induced by diethynitrosamine (DEN) in the rat. Moreover, it has been demonstrated the functional mitochondria maintenance and recovery with the compound treatment.

Because the IFC-305 beneficial effects on the mitochondrial function, we decided to evaluate the effect IFC-305 on autophagy in vivo in the sequential model of cirrhosis-HCC and if this compound is able to promote autophagy in vitro in MEFs. Experimental groups were formed considering a prevention scheme (DEN and IFC-305 administered the same weeks) and a reversion one (the IFC-305 treatment was given after the DEN administration was stopped).

The related-autophagy proteins Beclin, p62 and LC3 were reduced in the DEN-treated rats and the treatment with IFC-305 increased them. Moreover, PINK1, was increased with DEN while the IFC-305 treatment reduced it, suggesting the recuperation of ψm or mitophagy of abnormal mitochondria suggested by the diminution of parkin, because these proteins are in charge for labeling mitochondria with low membrane potential (ψm).

To corroborate the IFC-305 effects on autophagy, we decided to evaluate if this effect is reproduced in (mouse embryonic fibroblasts) MEFs isolated from mouse and treated with IFC-305 5 mM. It was observed a significant increment in Beclin p62 and LC3 with a maximum peak at 4 h. These effects matched with the autophagosome formation satined with Cyto ID.

These results suggest the IFC-305 ability to induce the autophagy process supporting its ability to avoid the accumulation of abnormal mitochondria and to its chemopreventive effect in the sequential model of cirrhosis-HCC induced by DEN.

THE ROLE OF OXIDATIVE STRESS AND GENDER IN THE ERYTHROCYTE ARGININE METABOLISM AND AMMONIA MANAGEMENT IN PATIENTS WITH TYPE 2 DIABETES

Martha L. Contreras-Zentella[1], Lourdes Sánchez-Sevilla[1], Marisela Olguín-Martínez[1], Martha G. Alatriste-Contreras[2] and Rolando Hernández-Muñoz[1].
[1]Departamento de Biología Celular y Desarrollo, Instituto de Fisiología Celular; UNAM. [2]División de Estudios Profesionales, Facultad de Economía, UNAM.
Martha L. Contreras-Zentella, Departamento de Biología Celular y Desarrollo, Instituto de Fisiología Celular; UNAM, Apdo. Postal 70-243. Coyoacán, 04510. Tel: +52 55 56225666; E-mail: mcontre@correo.ifc.unam.mx

ABSTRACT

Diabetes mellitus (DM) is a worldwide disease frequently associated with a high risk of atherosclerosis and renal, nervous system, and ocular damage. Reactive oxygen species (ROS) have been implicated in the pathogenesis of DM, as well as in its complications. Patients with type 2 DM frequently have vascular endothelium dysfunction, which is related to hypercholesterolemia, and nitric oxide (NO) deficiency, a major factor that contributes to endothelial dysfunction. Another source of possible complications in DM patients could be derived from an altered nitrogen metabolism, even in the absence of evident liver disease and/or nephropathy. The red blood cells (RBC) play an important role in vascular function, minimizing NO scavenging, and further delivering NO bioactivity in hypoxia, through the compartmentalization of hemoglobin (Hb). In fact, reduced levels of NO derived from arginine has been implicated in the vascular dysfunction of diabetic patients. An increased activity and expression of arginase I is associated with diabetes-induced increase in oxidative stress. Moreover, citrulline could promote NO production and endothelial function and improve peripheral insulin sensitivity, ameliorating organ perfusion and the endothelial metabolism, which might involve an antioxidant property.

We studied the differences in the levels of nitrogen metabolites and the correlations existing among them in both red blood cells (RBCs) and serum, as well as the possible differences by gender in healthy subjects and patients with type 2 DM. Healthy female and male controls showed a differential distribution of blood metabolites involved in arginine metabolism. Furthermore, most of the DM-induced alterations in nitrogen-related metabolites appear to be associated with a difference in the RBC capacity for the release of these metabolites, thereby causing an abrogation of the gender-related differential management of nitrogen metabolites in healthy subjects.

Conclusions: We found evidence of a putative role of RBC as an extra-hepatic mechanism for controlling serum levels of nitrogen-related metabolites, which differs according to gender in healthy subjects. Type 2 DM promotes higher ammonia, citrulline, and MDA blood levels, which culminate in a loss of the differential management of nitrogen-related metabolites seen in healthy women and men.

Cloning and biochemical characterization of three Glucose-6-Phosphate Dehydrogenase mutants presents in the Mexican population.

Yadira Yazmín Cortés-Morales[1], Saúl Gómez-Manzo[1]. Jaime Marcial-Quino[2] Alarcon-Zendejas Ana Paula[1], Figueroa-Rodríguez Vania Lorena[1], Erick Alcaraz-Carmona[1], Fabiola Fernández-Rosario[1], Víctor Martínez-Rosas[3], Laura Eloísa Morales-Luna[1], Edson Jiovany Ramírez-Nava[1], María Fernanda Torres-Rojas[1], Abigail González Valdez[4] and Beatriz Hernández Ochoa[5].

[1]Instituto Nacional de Pediatría. Laboratorio de Bioquímica Genética. SS,CD.MX, Mexico.
[2]CONACYT-Instituto Nacional de Pediatría, SS, CD.MX.Mexico.
[3]Escuela Nacional de Ciencias Biológicas, IPN, CD.MX, Mexico.
[4]Departamento de Biología Molecular y Biotecnología, Instituto de Investigaciones Biomédicas, UNAM, CD.MX, Mexico.
[5]Laboratorio de Inmunoquímica, Hospital Infantil de México Federico Gómez, CD.MX, Mexico.
E-Mail: yazbio9@ciencias.unam.mx; saulmanzo@ciencias.unam.mx

The deficiency of glucose-6-phosphate dehydrogenase (G6PD) is one of the most common inborn errors of metabolism worldwide. This congenital disorder generally results from mutations that are spread throughout the entire gene of G6PD. In this work, we reported for the first time the construction, cloning, overexpression, purification, and detailed functional and structural studies of three clinical variants: Class I G6PD Veracruz (Arg365His), Class II G6PD Seattle (Asp282His), and unclassified G6PD Mexico DF (Thr65Ala).

We found that all variants revealed a decrease in catalytic activity around 80 to 97% with respect to wild-type G6PD (WT) and had a decrease in affinity for both physiological substrates compared to WT G6PD. Furthermore, we found evidence that the mutations in the G6PDs mutants had a strong effect on the stability of these structure that exhibited significant differences at elevated temperatures. Our results also showed that the three mutations affected the three-dimensional structure of the protein, suggesting an unstable structure with low conformational stability that affected its G6PD functionality while the WTG6PD enzyme remained stable.

Finally, based on the biochemical characteristics and using the G6PD deficiency re-classification system proposed by Luzzato *et al*., 2016 we suggested that the unclassified G6PD Mexico DF could be a Class I G6PD variant considering only the biochemical characteristics obtained in this study, because it showed a loss of functional and structural parameter similar to other Class I mutants (Zacatecas, Durham, Nashville, Volendam and Andalus) as previously reported.

NaStEP stability in pollen tubes accounts for self-incompatibility in *Nicotiana alata*

Yuridia Cruz-González Zamora[1], Edgar Nájera-Torres[1], Lilia Angélica Bernal-Gracida[1], Javier Andrés Juárez-Díaz[2] Jimena Bellido Mora[1] and Felipe Cruz-García[1].
[1].- Departamento de Bioquímica, Conjunto E, Facultad de Química, Ciudad Universitaria, México CP.04510.
[2].- Departamento de Biología Comparada, Facultad de Ciencias, UNAM. Ciudad Universitaria, Cd. Mx. 04510.
e-mail: fcg@unam.mx

Self-incompatibility (SI) is the genetic mechanism by which some angiosperms accept or reject cross-pollen or self-pollen, respectively. In *Nicotiana alata*, the polymorphic *S*-locus controls SI. This locus encodes the S-RNase (female determinant), and SLF (male determinant). Their interaction inside the pollen tube dictates if the pollen tube (PT) reaches the ovary. However, there are other proteins, encoded outside the *S*-locus, that are known to be essential for SI: 120K, HT-B, and NaStEP.
NaStEP (*N. alata* Stigma Expressed Protein) is a Kunitz-type protease inhibitor localized only in the stigma´s exudate of SI *Nicotiana* species. Loss of function assay demonstrated NaStEP is essential for the SI response. HT-B is a small, rich asparagine style expressed protein that enters the PT. During pollination, HT-B localizes in the periphery of the vacuole that contains the S-RNase; its function is still unknown. Degradation of HT-B occurs inside the PT of a compatible cross, unlike an incompatible cross where it possibly participates breaking the vacuole releasing the S-RNases towards the cytoplasm.
The absence of NaStEP in RNAi transgenic lines, provokes the HT-B degradation regardless of the type of cross (compatible or incompatible), which indicates it plays a role in the stability of HT-B. In the present work, we studied NaStEP stability during compatible crosses by immunoanalysis and immunolocalization assays. We reestablished the SI system in transgenic NaStEP::RNAi lines by adding native NaStEP on stigmas of SI *N. alata* before pollination. We also tested if NaStEP protease activity is essential in the SI response by incorporating a recombinant NaStEP with its protease reactive site mutagenized, into NaStEP:: RNAi lines.

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Effect of heavy metals on *Ustilago maydis* respirasome

Jaime Abraham de Lira Sánchez[1], Mercedes Esparza Perusquia[1], Ricardo Jasso Chávez[2], Federico Martínez[1], Oscar Flores Herrera[1*].
[1]Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México.
[2]Departamento de Bioquímica, Instituto Nacional de Cardiología —Ignacio Chávez". *Tel.: 5623-2168 oflores@bq.unam.mx.

Metals and metalloids affect biological systems either as promote protein structure and function, or harmful when they are in excessive concentration (1, 2). Heavy metals toxicity is caused by a continuous exposition to environmental pollutants in soil, water and even air (3). Therefore, this chronic exposition in human, is considered a cause of pathologies associated to ROS production, DNA mutations, nutrient assimilation and protein function or activity alteration (2, 4, 5). Also, it is well know that mitochondria is sensitive to heavy metals and an increase of ROS occurs through respiratory complexes (6); however, its effect on the respirasome haven't be determined. The aim of this work was to evaluate the effect of cadmium, chromium, iron and cupper on the digitonin-solubilized respirasome from *Ustilago maydis*. The respirasome and free-CI were obtained according to the method described by (7). Mitochondria were obtained from *U. maydis* FB1 wild strain by a standard protocol and respiratory complexes and supercomplexes solubilized with digitonin (7). Solubilized complexes were loaded in a 0.5 - 1.5 M sucrose gradient, fractionated and analyzed in a 4 - 10% BN-PAGE to determine the fractions with pure respirasomes or free-CI. NADH:DBQ oxide-reductase activity from respirasomes was evaluated spectroscopically at 340 nm according to (8) in presence of 200 μM NADH, 600 μM DBQ and 16 μM cytochrome c. Free-CI activity was measured in similar experimental conditions. Finally, the effect of $K_2Cr_2O_7$, $FeCl_3$, $CdCl_3$ or $CuSO_4$ was achieved with a dose-response curve (0.1 – 3000 μM). Our results showed an IC_{50} of 200 μM for Cr, 300 μM for Cu, 400 μM for Cd, 400 μM for Fe on the *U. maydis* respirasome; while for free-CI, an IC_{50} of 100 μM for Cr, 100 μM for Fe, 200 μM for Cu and 1 mM for Cd. We conclude that the interaction of Complex I with the complexes III_2 and IV in the respirasome could contribute to resistant of CI against heavy metal inhibition compared to free-CI, at least by Fe, Cu and Cr.

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C-terminal amidation - Identification and expression of the amidating enzyme PAM from Scorpion *Centruroides noxius*

Gustavo Delgado Prudencio[1], Baltazar Becerril Lujan[1], Lourival Domingos Possani Postay[1], Ernesto Ortiz Suri[1*]
[1]Departmento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Avenida Universidad, 2001, Colonia Chamilpa, Cuernavaca, Morelos, 62210, México.
*erne@ibt.unam.mx, +52 (777) 311-4900 ext 247

α-amidation is one of the most important post-translational modifications that occurs in the cell. Many secreted peptides as neuropeptides, hormones and toxins require to be amidated for their correct biological function. This post-translational modification occurs on a glycine residue at the C-terminal of the precursor, and involves the sequential action of two enzymes: peptidylglycine α-hydroxylating monooxygenase, PHM; EC 1.14.17.3 and peptidyl-alpha-hydroxyglycine α-amidating lyase, PAL; EC 4.3.2.5. In some insects such as *Apis mellifera* and *Drosophila melanogaster* PHM and PAL are encoded in separate genes; in contrast, in mammals such as *Rattus norvegicus* and *Homo sapiens,* a single gene encodes the enzyme peptidyl-glycine α-amidating monooxygenase (PAM), a bifunctional enzyme that includes the catalytic domains PHM and PAL and a membrane spanning domain in the same polypeptide. Although amidating enzymes are widely distributed in eumetazoans, PAM, PHM and PAL are not present in plants, fungi or prokaryotes. The heterologous expression of amidated peptides in classical models such as bacteria, frequently requires the coexpression of an amidating enzyme, being the PAM of *Rattus norvegicus* the most used. The objective of this work is to explore the enzymatic amidation system present in the venom glands of scorpions. These arthropods are known to produce potent venoms, which contain a wide variety of amidated peptides (*e.g.* toxins and antimicrobial peptides). Sequences of bifunctional PAM and monofunctional PHM and PAL were identified from the transcriptomic analysis of 20 scorpion species from the new and old world. The PAM sequence of the Mexican scorpion *Centruroides noxius* (whose amidated toxin, Cn2, is the most abundant peptide in its venom) was amplified and cloned in the plasmid pBluescript II KS (+), the catalytic domains (PHM-PAL) were sub-cloned in the plasmid pPICZαA for its expression in *Pichia pastoris*. The use of the scorpion enzyme could be an alternative for *in vitro* amidation of peptides.

Key words: Amidated peptides, α-amidation, amidating enzyme PAM, *Centruroides noxius*

Isolation and characterization of the N66 protein from the mother oyster pearl shell *Pinctada mazatlanica*.

Rosa Virginia Dominguez Beltran, Crisalejandra Rivera Pérez, Reyna de Jesús Romero Geraldo, Norma Y. Hernández Saavedra.
Laboratorio de Genética Molecular, Centro de Investigaciones Biológicas del Noroeste S.C. (CIBNOR), Avenida Instituto Politécnico Nacional No. 195, Playa Palo Alto de Santa Rita, Apartado Postal 128, La Paz 23096, Baja California Sur, México. Tel: 1238484 ext. 3805, nhernan04@cibnor.mx

Biomineralization is a genetically regulated biological process responsible of mineralized tissues or biominerals such as bones, shells and coral skeletons, which provide structural support and protection. In mollusk, the shells are mainly composed of two different calcium carbonate (CaCO3) layers known as the outer prismatic (calcite) and inner nacreous (aragonite) layers, being nacreous layer highly resilient, it provides strength and toughness, due to its highly organized structure. Biomineralization requires several components such as calcium ion, bicarbonate and organic macromolecules that constituent organic matrix of the shell, which is composed mainly by polysaccharides, glycoproteins, proteins and β-chitin. Proteins involved in the biomineralization are synthesized in mantle of the mollusks and secreted into the extrapaleal space between shell and mantle. N66 is a protein expressed in the dorsal region of the mantle, which is known to be responsible of the formation of the layer nacreous, while in the edge are responsible of the formation of the prismatic layer, it has been described as a protein involved in nacre formation. In this work, we isolated and characterized biochemically the native N66 protein from the mother oyster pearl shell *Pinctada mazatlanica* in order to understand its role in the process of nacre formation. The native N66 protein from the mother oyster pearl shell (*Pinctada mazatlanica*) showed to be a soluble glycoprotein, with calcium binding capability, it exhibits carbonic anhydrase activity and it is able to form carbonate calcium crystals *in vitro*, calcite and aragonite, similar to those observed in the mother oyster pearl shell. This results suggest that N66 have a role in the calcification of carbonate calcium polymorphisms, which is modulated by the salts and cofactors used.

Biosynthesis of ATP in mitochondria from skeletal and cardiac muscle: Effect of metabolic syndrome and aging

Mohammed El-Hafidi, Miguel Ángel Barrios Maya, Ocarol López Acosta and Angélica Ruiz Ramírez.
Depto. de Biomedicina Cardiovascular, Instituto Nacional de Cardiología Ignacio Chávez, Tlalpan, CD México, Tel 55 73 29 11 Ext 25301, email: medelhafidi@yahoo.com

In recent decades, it has been proposed that mitochondria suffer oxidative stress and dysfunction during aging. Oxidative damage occurs selectively in the components of the electron transport chain whose constitutive peptides are encoded in mitochondrial DNA. However, mitochondrial theory of aging requires evidence that show a decrease in the production capacity of ATP in mitochondria of experimental models of aging. During aging the uncoupling oxidative phosphorylation and a decreased ATP synthesis may be related to the proton leak through mitochondrial inner membrane induced by free fatty acids (FFA) or by over-expression of uncoupling proteins (UCPs) found increased in metabolic disease. Metabolic syndrome (MS) is characterized by FFA accumulation and by increased uncoupling proteins in response to oxidative stress and lipotoxicity in several tissue. Therefore, the objective of the present work was to evaluate the effect of lipid metabolism alterations in combination of aging on the biosynthesis of ATP in skeletal and cardiac muscle from an animal model of MS induced sucrose diet during 6, 12, 24 and 56 weeks.
The results show that fat accumulation in the intra-abdominal cavity of rats treated with sucrose (MS) appeared from 6 weeks of age. However, the levels of triglycerides in MS was started to increase significantly from the week 12. From 24 weeks of age and treatment, rats show a complete development of MS (obesity, hypertriglyceridemia and hypertension). Regarding the activity of the ATP synthase, a significant decrease in the activity was started to be obvious just from week 24 in mitochondria from skeletal muscle. Nevertheless, in mitochondria from cardiac muscle no difference in the activity of ATP synthase was observed between both groups and at any age. The activity of ATP synthase in both tissues was evaluated in the presence of oleic acid known for its uncoupling effect. It was observed that the synthesis of ATP is sensitive to oleic acid and this sensitivity decreases in the presence of GDP a physiological inhibitor of UCPs. Our results show that the activity ATP synthase in mitochondria from skeletal muscle with MS is reduced and is more sensitive to the uncoupling effect of oleic acid than mitochondria from cardiac muscle and this sensitivity to oleic acid increases with age.

Characterization of the triosephosphate isomerase from *Encephalitozoon intestinalis*. Proposal of a new pharmacological target.

Sergio Enríquez Flores[1], Itzhel García Torres[1], Ignacio De la Mora De la Mora[1] y Gabriel López velázquez[1].
[1] Instituto Nacional de Pediatría. Lab. EIMyT - Grupo de Estudio de Biomoléculas y Salud Infantil. 5º Piso, Torre de Investigación.
Av. Insurgentes sur 3700-C. Col. Insurgentes Cuicuilco, Del. Coyoacán, C.P. 04530, Ciudad de México.
Tel. 10840900 ext. 1726; sergioenr@gmail.com

Abstract

Encephalitozoon intestinalis is the second microsporidia most frequently associated with gastrointestinal disease in humans, especially immunocompromised or immunosuppressed individuals, including children and the elderly population. The prevalence reported worldwide in these groups ranges from 0 to 50 %. Currently, albendazole is the drug most commonly used to treat microsporidiosis caused by *Encephalitozoon* species. However, the results of treatment are variable, and relapse can occur. Thus, efforts are being directed toward identifying more effective drugs for treating the microsporidiosis disease, and the study of new molecular targets appears promising. *E. intestinalis* lack of organelles such as mitochondria, so that oxidative phosphorylation does not occur, which suggests that the glycolysis is important to generate ATP and other metabolites, therefore the enzymes involved in such pathway are attractive drug targets.
Based on the above, we characterized the glycolytic enzyme triosephosphate isomerase of *E. intestinalis* (EiTIM) at the functional and structural levels. Our results demonstrate that the EiTIM is an enzyme catalytically competent with kinetic parameters very similar to other TIMs. Interestingly, EiTIM is efficiently inactivated by sulfhydryl reagents and we demonstrate the effects of different commercially approved drugs on the inactivation of EiTIM via derivatization of its Cys residues. The most striking result of this study is the demonstration that established safe drugs such as omeprazole, rabeprazole and sulbutiamine can effectively inactivate this microsporidial enzyme and might be considered as potential drugs for treating this important disease.

The conformational landscape of proteins: study of *de novo* designed TIM barrels

Sergio Romero-Romero,[1],* Daniel-Adriano Silva Manzano,[2] Miguel Costas,[3] Sooruban Shanmugaratnam,[4] Birte Höcker,4 David Baker,[2] and D. Alejandro Fernández-Velasco[1],*

[1]Facultad de Medicina, UNAM, Mexico, [2]Institute for Protein Design, Washington University, USA, [3]Facultad de Química, UNAM, Mexico, [4]Lehrstuhl für Biochemie, Universität Bayreuth, Germany.
* Corresponding authors: Laboratorio de Fisicoquímica e Ingeniería de Proteínas. Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México. Ciudad Universitaria, 04510, México. Phone numbers: (+521)-5556232259.
E-mails: sergioale152@gmail.com (SRR), fdaniel@unam.mx (DAFV).

Protein scientists are paving the way to a new phase in protein design and engineering. Specifically, we are focused on the problem of *de novo* protein design: the generation of new proteins on the basis of physical principles with sequences unrelated to those in nature. The TIM barrel topology is one of the most robust, versatile and abundant in nature, for this reason, it has been through the years a central goal for the design and engineering of proteins. sTIM11, the first successful *de novo* designed TIM barrel was described previously. sTIM11 presents a low conformational stability and free cysteines. The aim of this work was to increase the stability of sTIM11 in order to use this scaffold for molecular functionalization. 28 new proteins (named ReTIMs) were designed to increase the packing in three different regions of the barrel and to introduce favorable interactions. All the designs were overexpressed and purified to homogeneity. The thermal and chemical-induced unfolding of these proteins was studied by differential scanning calorimetry, circular dichroism and intrinsic fluorescence. Three of these proteins had improved characteristics compared to sTIM11: ReTIM17 (Tm: 107.2 °C and DG at 25 °C: 6.5 kcal/mol), ReTIM18 (Tm: 92.9 °C and DG at 25 °C: 4.1 kcal/mol) and ReTIM24 (Tm: 117.3 °C). The stability curves of the proteins with reversible thermal unfolding showed that the shape of the curve is modulated by Ts (temperature where DS=0), DH (enthalpy value in TS), and DCp. Structural analysis of ReTIM18 shows that the stabilizing interactions were correctly designed (RMSD crystal structure vs Rosetta model is 0.87A). The best design has the stability 50 percent increased and Tm value 42 degrees higher than sTIM11. Finally, the main effect of varying the aminoacidic sequence is in the Tm value and not on the stability at room temperature.

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Deletion of the complex V-dimerizing subunit *g* and its effect on the mitochondrial bioenergetics in *Ustilago maydis*

Mercedes Esparza-Perusquía, Sofía Olvera-Sánchez, Juan Pablo Pardo, Federico Martínez and Oscar Flores-Herrera. Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México. Circuito Escolar, Cd. Universitaria. UNAM 04510, México, D.F. Tel.: 5623-2168 oflores@bq.unam.mx.

Mitochondrial F_1F_0-ATP synthase is an enzymatic complex involved in the aerobic synthesis of ATP. It is well known that several enzymes are organized in supramolecular complexes in the inner mitochondrial membrane. The supramolecular ATP synthase assembly is mediated through two interfaces. One leads to dimer formation and the other to oligomer formation. The F_1F_0-ATP synthase synthetizes more than 90% of cellular ATP coupled to the proton electrochemical potential ($\Delta\mu_{H+}$).
Characterization of the DDM stimulated ATPase activity of V_2 and V_1 from *Ustilago maydis* showed that isolated V_2 is 9-times more active that isolated V_1[1]. This observation highlights the role of the interphase in the activity of dimer. It has been reported that subunits g and e are involved in the interphase and its deletion decrease dimer concentration and modified the cristae architecture[2]. In this work the deletion of g-subunit was performed and its effect in the mitochondrial bioenergetics was determined.
Elimination of *g* subunit in *U. maydis* didn't prevented complex V dimerization; moreover, in a BN-PAGE the monomer/dimer ratio was similar between wild type strain and Δ*g* mutant. However, spectrophotometric analysis of the ATPase activity of the isolated dimer showed that it was slower than the monomer, suggesting that *g* subunit is not essential for dimerization but it could have an important role in the activity of the enzyme. Interestingly, Δ*g*-strain express the AOX in the early stages of growth, suggesting an oxidative stress produced, probably, by complex I and III[3].
Ultrastructure analysis of mitochondrial architecture of Δ*g*-strain from *U. maydis* showed that cristae were tubular and lamellar, and no as onion rings described for Δ*g* mutant of *S. cerevisiae*. Likewise, ATP synthesis and $\Delta\Psi_m$, in the Δ*g* and WT-strain were similar; suggesting that, although ATPase activity of dimer was suppressed, its role in cristae structure and bioenergetics was intact[3].

1) Esparza-Perusquía *et al.,* 2017. 2) Paumard *et al.,* 2002. 3) Esparza-Perusquía *et al.,* en preparación.

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Atomic structure of Ornithine Decarboxylase from *Saccharomyces cerevisiae*: Solving the mechanism of its dual activity

Jessica Georgina Filisola Villaseñor, Viridiana Olin Sandoval, Edgar Morales Ríos. CINVESTAV Zacatenco, Av. IPN No. 2508, San Pedro Zacatenco, CP 07360, CDMX, México. Tel. 57473800 Ext. 5234

Polyamines (PAs) are ubiquitous aliphatic polycations. The most common PAs are: spermidine (Spd), spermine (Spm), putrescine (Put) and cadaverine (Cad). PAs are involved in several cellular processes such as transcription, translation, stress response, growth, etc.(1). The synthesis of PAs begins with the decarboxylation of ornithine (Orn) by Ornithine Decarboxylase (ODC) forming Put, followed by the addition of aminopropyl groups to form Spd and Spm by Spd and Spm synthases (SpdS and SpmS), respectively(1). Moreover, *Saccharomyces cerevisiae* can transport PAs across the plasma membrane with at least ten transporters (1).

A response mechanism to oxidative stress in *S. cerevisiae* involves the export of Spd and Spm by the Tpo1 transporter (2). It has also been identified that three enzymes of the lysine (Lys) biosynthesis pathway (Lys2, Lys9 and Lys12), increase their expression in response to oxidative stress when Tpo1 is absent. This suggests that there is a relationship between the metabolism of Lys and PAs (3). In bacteria, cyanobacteria and some plants, Lys is decarboxylated to Cad by lysine decarboxylase (LDC); however fungi and animal cells lack this gene. Recently, it was identified that, in *S. cerevisiae*, Cad is synthesized by an alternative activity of ODC (ScODC), and that this reaction has an important role in maintaining the homeostasis of Lys in response to oxidative stress(2, 3).

ODC has been structurally described in several organisms. However, although ScODC can metabolize both substrates under physiological conditions, there are not crystal structures that describe the atomic mechanism of this duality. Therefore, the aim of this work is to structurally characterize ScODC by X-ray crystallography, in order to understand the mechanism of catalysis with both ligands. So far, we have cloned, overexpressed and purified the recombinant protein. Also, we have produced non-diffracting crystals. We are now performing the crystal optimization by adding Cad, D-Lys and Put, so that we can carry out diffraction analysis of these crystals using synchrotron X-rays and thus, obtain the atomic structures that will allow us to understand the mechanism of catalysis of this enzyme with both substrates.

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Effect of heavy metals on the ATPase activity of the V_2 and V_1 from *Ustilago maydis*

Giovanni García-Cruz, Mercedes Esparza-Perusquía, Sofía Olvera-Sánchez, Federico Martínez, Juan Pablo Pardo, Oscar Flores-Herrera*. Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México. Tel.: 5623-2168 oflores@bq.unam.mx.

Continuous exposition to heavy metals from environmental pollution is cause of many diseases associated to ROS overproduction (1). It is well know that heavy metals induce and increase of ROS in mitochondria through respiratory complexes (2) with a drop in ATP synthesis. However, the effect of heavy metals on the dimer (V_2) of F_1F_0-ATP synthase hasn't be determined. The aim of this work was to evaluate the effect of heavy metals on the digitonin-solubilized V_2 from *Ustilago maydis*. The V_2 and V_1 were obtained according to the method described by (3). Briefly, *U. maydis* FB1 wild strain was growth in YPD medium during 24 h at 28°C and mitochondria were obtained by a standard protocol and respiratory complexes and supercomplexes solubilized with digitonin (3). Solubilized complexes were loaded in a 0.5 - 1.5 M sucrose gradient, fractionated and analyzed in a 4 - 10% BN-PAGE to determine the fractions with V_2 or V_1. ATPase activity from isolated V_2 or V_1 was evaluated spectroscopically as NADH oxidation *(ε_{NADH} = 6.22 $mM^{-1}cm^{-1}$ at 340 nm)* using an enzymatic coupling reaction with the pyruvate kinase and lactate dehydrogenase (3). The effect of $K_2Cr_2O_7$, $FeCl_3$, $CdCl_3$ or $CuSO_4$ was achieved with a dose-response curve (0.1 – 3000 μM).

Our results showed a 90% decrease of ATPase activity from V_2 and V_1 using cadmium in range concentration of 500 μM to 1500 μM, in this zone the activity of ATPase was inhibited completely., our aim is find the concentration on dose-response curve in which activity show the IC50 from V_2 and V_1

We conclude that the presence of cadmium significantly affects the activity of complex V_2 and V_1 from *U. maydis*, where 500 μM of Cd it´s enough to inhibited totally ATPase activity.

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Non-classical Hydrophobic Effect In The Recognition Of Hydrophobic Ligands By Bovine Odorant Binding Protein

Enrique García Hernández and Homero Gómez Velasco
Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, México 04510, D.F., México.

Odorant binding protein (OBP) belongs to lipocalins, a large family of structurally related protein carriers whose ligand binding site is defined by a hydrophobic pocket located inside a nine β-strand barrel. OBP is atypical among lipocalins because it forms dimers stabilized through intercatenary domain swapping. Although it has been proposed that OBP specializes in transporting odorants and as a scavenger for residual hydrophobic molecules in the nasal epithelia of mammals, the actual biological function of OBP is still controversial. In this work, we explore the energetic determinants of the affinity of bovine odorant binding protein (OBP) for its natural hydrophobic ligand octenol. Differential scanning calorimetry measurements revealed that the native state of OBP is composed of a slow equilibrium of free and dimerized subunits. The conformational stability of the monomeric subunit is very similar to that exhibited by an OBP mutant engineered to fully monomerize. Isothermal titration calorimetry measurements showed that OBP and the obligated monomer bind to octenol with very similar thermodynamic signatures, characterized by a submicromolar affinity driven only by a large enthalpy component. Further characterization of ligand binding as a function of temperature allowed the determination of kinetic and equilibrium parameters, which resulted consistent with a largely pre-dried OBP binding site. Nevertheless, molecular dynamics simulations of OBP revealed the presence of several water molecules in the protein binding cavity whose expulsion upon ligand binding contributes considerably to the overall enthalpy change. Furthermore, the simulations showed that OBP has a complex conformational behavior, forming multiple transient orifices through which the water molecules and the ligand can penetrate/leave the protein interior in a time scale of tens of nanoseconds. Together, our results indicate that the ~~-a~~nomalous" enthalpy that drives OBP binding towards hydrophobic ligands originates from a lower desolvation enthalpy cost and the return of high-energy water molecules to bulk water.

Hypoxia effects on matrix metalloproteinases expression in lung cancer.

Antonio Armando García Hernández, Cuauhtémoc Sandoval Sandoval, Bettina Sommer Cervantes, Ramses Falfán Valencia, Arnoldo Aquino Gálvez, Georgina González Avila.
Biomedical Oncology Laboratory, National Institute of Respiratory Diseases –Ismael Cosío Villegas"
Calz. de Tlalpan No. 4502, Del Tlalpan, Col. Section XVI, Mexico City, C.P. 14080.
Head of the group. Dra. González Avila. Tel. 54871700 ext 5287.
ggonzalezavila@yahoo.com

Rapid tumor growth causes hypoxia inside them. This change in tumor microenvironment can favor neoplastic cells migration and angiogenesis in which matrix metalloproteinases (MMPs) have an important role. The objective of this work was to determine hypoxia effects on MMPs expression and enzymatic activity.

Material and methods.
Lung adenocarcinoma cells (A-549) were cultured under hypoxic conditions (1% O2) for 24, 48, and 72 hours. Cells that remained in normoxia for each period were considered as controls. After cell incubation, culture medium was taken and MMP-2 and MMP-9 enzymatic activity was evaluated by zymogram while their protein expression was assessed by Western Blot.

Results.
In hypoxic cells, an increase in MMP-2 and MMP-9 protein expression was found, especially at 48 h. MMP-14 levels were also increased in hypoxic conditions at 48 h. On the contrarily, free TIMP-2 decreased, showing bands that corresponded to activation complexes (proMMP-2/MMP-14/TIMP-2) at the same exposure times; these bands were also observed in MMP-2 Western blot results. MMP-3 concentration was increased in hypoxia conditions at 24 and 48 h. Free TIMP-1 decreased at 48 h in hypoxia, showing high molecular weight bands that could correspond to activation complexes (proMMP-9/MMP-3/MMP-9).
On the other hand, the highest gelatinolytic activity was observed at 48 h under hypoxia conditions. The enzymatic activity of MMP-2 in hypoxia was 43.476.5±9294.1 densitometry units (DU), in comparison with normoxic conditions (18.861.23 ± 7304.94 DU), ($p = 0.008$). MMP-9 behaved similarly at 48 h (hypoxia = 33.314.22 ± 4809.89 DU, normoxia = 24.940.7 ± 3815.8 DU, $p = 0.03$).

Conclusions.
Hypoxia has effects on MMPs protein expression. The increase in MMP-14 expression under hypoxia conditions could favor the formation of proMMP-2 activation complexes while MMP-3 increased synthesis may be also involved in MMP-9 activation which explains the high activity of these enzymes under hypoxic conditions.

Analysis of *in vivo* interaction of Ribonuclease II, Ribonuclease PH, and RNA Degradosome in *Escherichia coli*.

Noé Arroyo-Velez, Marcos Emmanuel Jaso-Vera, and Jaime García-Mena.
Departamento de Genética y Biología Molecular, Cinvestav Unidad Zacatenco. Av. Instituto Politécnico Nacional 2508, Col. San Pedro Zacatenco, CDMX 07360. México. (55) 5747-3800 X 5328, jgmena@cinvestav.mx

In *Eschericha coli* the RNA degradosome (RNAD) accomplish most of the regulated degradation of RNA. Even though RNAD is assembled by interaction of Ribonuclease E (RNase E) with canonical components like RhlB helicase, polynucleotide phosphorylase (PNPase), and Enolase, RNase E enzyme is also capable to interact with other proteins, mainly ribonucleases like RNase PH and RNase II thus modifying its structure, function and specificity for certain RNAs. Interactions between canonical (RNase E) and non-canonical components of the RNAD (RNase II and RNase PH), have been proved previously *in vitro* however it is important to corroborate such interactions *in vivo*.
In this work we evaluate the interactions between Ribonuclease E and Ribonuclease II and Ribonuclease PH *in vivo* using Förster resonance energy transfer analysis (FRET). To achieve this goal, we constructed *Escherichia coli* strains carrying chromosomal fusions of native RNase PH or RNase II, fused to the fluorescent protein phi-Yellow (phi-YFP) which functioned as fluorescence acceptor and RNase E protein fused to the Cyan Fluorescent Protein (CFP) that works as a donor of fluorescence using the recombineering method for prokaryotes. A negative control that cannot assemble a degradosome due to a C-terminal in Ribonuclease E (RNaseE-701), was also constructed. All constructions were confirmed by PCR of recombined genes and fluorometric assays.
The energy transfer between fluorescent proteins fused to the ribonucleases was measured *in vivo* by end-point Fluorometric assay using Synergy H4 Hybrid Reader (BioTek) in *E. coli* grown overnight in LB medium, and the apparent FRET efficiency (FRET app) was calculated. We detected low levels of fluorescence for the phi-YFP fluorophore fused to the phi-YFP protein (RNase II and RNase PH), which could be due that the ribonucleases are transcribed from its cognate promoters, and present low levels of expression. Aside of this, we could obtain results that suggests *in vivo* interaction between Ribonuclease E-Ribonuclease II, and between Ribonuclease E-Ribonuclease PH. Further analyses are needed to better characterize these interactions and to confirm the low level of expression of RNase II and RNase PH.

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Glucose-6-phosphate dehydrogenase (G6PD) deficient; a biochemical perspective

Saúl Gómez-Manzo[1], Jaime Marcial-Quino[2], Marín Gutierrez-Alejandra Guadalupe[1], Pérez-Acosta Alegna Monserrat[1], Edson Jiovany Ramírez Nava[1], Yadira Yazmín Cortés Morales[1], Víctor Martínez Rosas[1], Laura Eloísa Morales Luna[1], Erick Alcaraz Carmona[1], Fabiola Fernández Rosario1, María Fernanda Torres Rojas[1], Abigail González Valdez[3], Paulina Hernández Ochoa[1], Beatriz Hernández Ochoa[4].
[1]Instituto Nacional de Pediatría. Laboratorio de Bioquímica Genética. SS, CD.MX.
[2]CONACYT-Instituto Nacional de Pediatría, SS, CD.MX.
[3]Departamento de Biología Molecular y Biotecnología, Instituto de Investigaciones Biomédicas, UNAM, CD.MX.
[4]Laboratorio de Inmunoquímica, Hospital Infantil de México Federico Gómez, CD.MX. Mexico. E-Mail: saulmanzo@ciencias.unam.mx

Abstract: G6PD deficiency is the most common enzymopathy, leading to alterations in the first step in the pentose phosphate pathway, which interferes with the protection of the erythrocyte against oxidative stress and cause a wide range of clinical symptoms being the hemolysis one of the most severe. The G6PD deficiency causes several abnormalities that range from asymptomatic individuals to more severe manifestations that could trigger the death. Recently, an extensive review has been published, recognizing 217 mutations in the *g6pd* gene that are responsible for the severity of the clinical symptoms. Nowadays only 9.2% of all recognized variants have been related with clinical manifestations.
It is important to understand the molecular basis of G6PD deficiency to known how gene mutations can impact the structure, stability and enzymatic function. In this work, we compare the functional and structural data that has been generated thought the characterization of 30 G6PD variants using different approaches. The methods to achieve the most representative behavior of the variant and the best kinetic parameters to compare between variants have been addressed as well as the analysis of the protein stability by different assays and tools. The impairment in the enzymatic activity of each variant seems to be related to alterations in the ligand binding site or the structural stability leading to a shorter half-life or misfolding of the protein. These studies showed that the severe clinical manifestations of G6PD deficiency are related to mutations affecting the catalytic site and the structural NADPH binding site, stating that misfolding of the protein or instability of the 3D structure of the protein could be compromising the half-life of the protein in the erythrocyte and its activity. This characterization is related with the clinical outcome, allowing a best understanding of the G6PD mutants and a concomitant management for the patients.

New structural insights regarding the binding mode of the Kunitz-type protease inhibitors: canonical or non-canonical subclassification.

Yasel Guerra[1,2], Colin Berry[3], Alejandro Fernández-Velasco[2], Enrique Rudiño-Piñera[1]
[1]Laboratorio de Bioquímica Estructural, Departamento de Medicina Molecular y Bioprocesos. Instituto de Biotecnología, UNAM. Av. Universidad #2001, Col. Chamilpa C.P. 62210. Cuernavaca, Morelos. Correo: yaselg@ibt.unam.mx Teléfono: 5528414996. [2]Laboratorio de Fisicoquímica e Ingeniería de Proteínas, Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, México [3]Cardiff School of Bioscience, Cardiff University, Wales, UK.

The Kunitz-type STI family is a large family of proteins most of the members of which are protease inhibitors. The versatility of the inhibitory profile of some members together with the applications of the protease inhibitors in biotechnology and biomedicine make this family a promising scaffold for designing new multifunctional proteins. Although, initially all Kunitz-type STI inhibitors were considered as canonical serine protease inhibitors, in recent years new non-canonical Kunitz-type STI inhibitors have been discovered. A special case is a double-headed inhibitor from *Sagittaria sagittifolia* which has both canonical and non-canonical serine protease binding loops. The different protease binding modes present in the Kunitz-STI family could be the result of different mechanisms of evolution in the β-trefoil fold, as described for other proteins with the same fold. One limitation to the classification of an inhibitor as canonical or non-canonical based only on its sequence is that the canonical classification relies on a structural basis. Even so, most authors tend to consider every new Kunitz-STI inhibitor as a canonical one and try to assign the serine protease binding loop between strands 4 and 5, through analogy with the representative protein of the family: the Soybean Trypsin Inhibitor (STI). However, preliminary work suggests that this assumption is not always valid. In the present work, we used structural bioinformatic analysis of the crystallographic structures of the Kunitz-type STI family members available in the PDB in order to help to sub-classify the inhibitors of this family according to their protease binding mode. Our results suggest that a species phylogenetic tree could be an effective tool for the classification of new inhibitors as canonical or not. Recently released three-dimensional structures of Kunitz-STI inhibitors were reanalyzed using our method, suggesting a different binding mode to that originally proposed for them. We consider that the methodology proposed in this work to sub-classify the Kunitz-STI will help in the identification of potential protease-binding loops for serine proteases.

Spectroscopic studies show evidence that curcumin derivatives inhibit TGR of *Taenia crassiceps* cysticerci

Alberto Guevara-Flores, José de Jesús Martínez-González, Álvaro Miguel Herrera-Juárez, Juan Luis Rendón e Irene Patricia del Arenal Mena*.
Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México.
Correspondencia: Facultad de Medicina, Ciudad Universitaria; Ciudad de México, CP 04510 Tel. +52(55) 5623-2169 *Correo electrónico: darenal@bq.unam.mx

Curcumin, also called diferuloylmethane (IUPAC name (1*E*, 6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), is a compound which has been widely studied due to its multiple biological effects. Particularly interesting is their effect as enzyme inhibitor. In aqueous media curcumin is highly unstable, leading to a variety of derivatives. The stability of the compound is dependent on factors such as pH, light, as well as the presence of other curcuminoids.

An interesting and potentially useful as drug target is the multifunctional thioredoxin-glutathione reductase (TGR) enzyme, an isoform of thioredoxin reductase. In parasite flatworms, the enzyme is responsible for the redox homeostasis and essential in the survival of these parasites. In this work the effect of pure and impure curcumin, as well as some of its degradation products on the GSSG reductase activity of TGR from *Taenia crassiceps* cysticerci was studied.

Through simultaneous spectroscopic and kinetic analysis, it is demonstrated that an auto-oxidation product of curcumin is able to inhibit the enzyme. Based on the absorption spectra of curcumin derivatives, spiroepoxide appears as the best candidate to explain the inhibition data. Further, six degradation products of curcumin were tested for its ability as TGR inhibitors. The order of inhibition power was: 4-vinylguaiacol (4-VG)>Feruloyl aldehyde>Feruloyl methane. The acid derivatives of curcumin Vanilic acid and Ferulic acid were poor inhibitors. Based on these results, it is proposed that curcumin derivatives can be considered as potential prodrugs against parasitosis caused by flatworms.

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Function of the Cox1 carboxyl terminal-end in yeast phenotype and mitochondrial function

Ana Paulina Gutiérrez-Alejandre, Yolanda Camacho-Villasana, Rodolfo García-Villegas, Norma Lilia Morales-García, Salvador Uribe-Carvajal and Xochitl Pérez-Martínez
Departamento de Genética Molecular, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Circuito exterior s/n, Ciudad Universitaria, Coyoacán, 04510 Ciudad de México. Tel. (0155) 5622 5662. Correo electrónico: pgutierrez@email.ifc.unam.mx

The mitochondrial genome of *Saccharomyces cerevisiae* codes for Cox1, Cox2 and Cox3, 3 of the 12 subunits that form the Cytochrome c oxidase *(C*c*O)*. This enzyme is the last electron acceptor complex in the respiratory chain, where electrons from cytochrome c to O2 are transfered. The rest of the subunits are encoded in the nuclear genome and imported from the cytosol into the mitochondria (1). Cox1 is a large and hydrophobic subunit that has 12 domains that cross the mitochondrial inner membrane and is part of the catalytic center of the C*c*O. Its carboxyl terminal-end (C-ter) is oriented towards the mitochondrial matrix. In recent studies, our laboratory has shown that the Cox1 C-ter is responsible for stabilizing Cox1, regulating its synthesis and supercomplexes formation/stability. Therefore, the Cox1 C-ter is a central regulator of the C*c*O biogenesis in a complex process that additionaly involves several chaperones (2, 3). We asked what was the physiological role of such an intricate regulation of C*c*O biogenesis was, and how loosing these mechanism affects cell physiology and mitochondrial function of yeast cells. The aim of the project is to characterize the phenotype and mitochondrial function of S. cerevisiae in the mutant strains of the C-ter of Cox1.

We observed that elimination of the last 15 amino acids of the Cox1 C-ter (Cox1ΔC15) decreased the chronological life span of cells. So far, we have not observed any defects on cell growth on different carbon sources or diminished resistance to H_2O_2 as expected for other C*c*O mutants (4).

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Biochemical characterization of *Rhinella marina* skin secretions and identification of enzymatic activities

Itzel Amairani Gutiérrez Reyna[1], Luis Caspeta Guadarrama[2], y Lidia González Morales[1].
[1]Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos.
[2]Instituto de Biotecnología, Universidad Nacional Autónoma de México. Av. Universidad No. 1001, Col Chamilpa, Cuernavaca, Morelos, México. C.P. 62209. Tel. 777 3297057. Email: lidia.gonzalez@uaem.mx.

Beginning with the secretions of anuran skin, a great variety of biological activities and the responsible molecules have been identified, the latter with medical-pharmaceutical potential. Due to this, it is important to continue with the search for this type of compounds in Mexican anuran species like *Rhinella marina*, whose skin secretions have not been explored for the characterization of novel molecules. Furthermore, it is also important to characterize the compounds found in this organism, since its secretions are successfully used in traditional Mexican medicine. In this work, we analyzed biochemical composition and enzymatic activities of skin secretions from *R. marina* collected in Colima state. With a reverse phase HPLC, the chromatographic profile of skin secretions was obtained. This suggested the presence of at least 24 distinct fractions with molecular weights ranging between 316 to 6448 Da. Using zymograms specific for detection of protease activity, we detected four components with this activity, with molecular weights corresponding to 70, 55, 40 and 30 kDa. The presence of phospholipase activity was also identified in plate assays. This is the first report focused on the characterization and identification of the components present in the secretions of the skin of Mexican toad *R. marina*.

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Biochemical characterization of HSL *BaEstB* esterase from *Bjerkandera adusta* and its natural substrates

Daniel Hernández Fuentes[1], Jorge Luis Folch Mallol[1], Alexandre Cardoso Taketa[1], Ramón Alberto Batista García[2], Nina Pastor[2], Edmundo Castillo[3], María del Rayo Sánchez Carbente[1].
[1]Centro de Investigación en Biotecnología, [2]Centro de Investigación en Dinámica Celular, [3]Instituto de Biotecnología, UNAM.
Universidad Autónoma del Estado de Morelos. Av. Universidad 1001 Col. Chamilpa, Cuernavaca 62209, Morelos, México. 777789 0260 danielhfuaem@gmail.com

ABSTRACT

White rot fungi have the ability to exploit all components of vegetable biomass due to the secretion of a variety of lignocellulolytic enzymes. The hydrolytic activities of these enzymes have a high potential for use in the paper and food industry and the production of bioethanol and biodiesel. *Bjerkandera adusta* is a basidiomycete that also has another type of enzyme belonging to the group of esterases and lipases widely used in industry and with high potential for biotechnological use.

In the laboratory of fungal molecular biology of the CEIB, the esterase HSL (hormone-sensitive Lipase) *BaEstB* obtained obtained from a *B. adusta* cDNA library grown in petroleum was biochemically characterized.This enzyme has the ability to hydrolyze substrates such as β-Naphthyl-Acetate and its activity is lower in the presence of substrates with longer carbon chains. However, until now it is unknown the natural substrates of this enzyme in the fungus. In mammals, HSL are involved in hormone-stimulated lipolysis of adipose tissue, the hydrolysis of diacylglycerol for the generation of ATP, as well as in the process of steroidogenesis. It is therefore important to study what are the substrates of the enzyme and whether the change of some amino acids in the substrate binding site could modify the specificity of the substrates used by *BaEstB*. The main objective of this work is to determine if *BaEstB* is able to deesterify cholesterol and ergosterol, as well as to analyze the modeling of the interaction between these substrates and the enzyme at the atomic level, by means of bioinformatics tools such as Docking, and finally to evaluate the substrate specificity of the mutant versions of *BaEstB*.

A new three-dimensional model of the *BaEstB* was obtained in which all the amino acid residues involved in the catalysis were identified. Preliminary results obtained with with obtain Docking modelling indicate that the enzyme interacts both with 2-Naphthyl Acetate and with Cholesteryl-acetate favorably (thebinding affinity energy between the substrate and the enzyme for the former with -7.8 kcal / mol while for second with-8.2 kcal / mol). This result suggest that the enzyme can hydrolyze the cholesteryl acetate, however it is necessary to carry out more analyzes of this type in which other substrates are included, such as p-NP Acetate and p-NP Butyrate and esterified ergosterol, that could be the enzyme substrate in *B. adusta*

PURIFICATION AND CHARACTERIZATION OF NOVEL METALOPROTEASES FROM *Holothuria inornata* FROM CHAMELA BAY, JALISCO, MEXICO

Alan G. Hernández-Melgar[1], Jorge A. Osorio-Kuan[1], Martha M. Manzano-Mora[1], Miguel Cuevas Cruz[1],Barbarín Arreguín-Lozano[1], Francisco Alonso Solís-Marín[2] & Roberto Arreguín-Espinosa[1]; AGHM: alan.hernandez@mail.com; RAE: arrespin@unam.mx
[1] Departamento de Química de Biomacromoléculas, Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Ciudad Universitaria, México 04510, Ciudad de México, México.
[2] Laboratorio de Sistemática y Ecología de Equinodermos, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Circuito Exterior S/N, 04510 Mexico City, Mexico

The proteases are a group of enzymes that are capable of hydrolize proteins and are related to a number of processes in all living organisms. These proteins are also important since they represent approximately 60% of global trade in enzymes (Mala B. *et al*, 1998), and have several applications in medicine, biotechnology, industry and research activities (Rani *et al.*, 2012).

The metalloproteinases or matrix metalloproteinases, is diverse family of zinc-dependent endoproteinases (has a metal ion of Zn2+ in the active site, hence the prefix ~~-m~~metallo") that are capable to degrade all the components that make up the extracellular matrix (López-Otín *et al*., 2010, López-Otín and Bond, 2008).

Echinoderms and in particularly sea cucumbers are an interesting group for the discovery of new proteases, since they are composed of collagen in its majority, have a high autolytic activity for the regeneration of organs and the ability of feeding on detritus. However, very little research have been focused in this issue, and only a few proteolytic enzymes have been reported; although no kynetic parameters have been defined. Therefore this research focuses on the isolation and kynetic characterization of novel metaloproteases form *Holothuria inornata* collected from Chamela bay in Jalisco, México.

Key words: Proteases, Metalloproteases, *Holothuria inornata*, purification of proteins,enzyme kynetics.

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IMMUNOPHENOTYPE OF SENESCENT CELLS

Elisa Hernández Mercado[1,5,6], Jessica Lakshmi Prieto Chávez[2], Lourdes Arriaga Pizano[2], Salomón Hernández Gutierrez[4], Fela Mendlovic[3], Mina Königsberg Fainstein[1], Norma Edith López Díaz Guerrero[1$].
[1]Lab.Bioenergética y Envejecimiento Celular, UAM-I, [2]Unidad Médica de Investigación en Inmunoquímica, Hospital Siglo XXI, IMSS, [3]Departamento de Microbiología y Parasitología, Facultad de Medicina, UNAM, [4]Departmento de Biología Molecular, Escuela de medicina, UP.
[6]Posgrado en Biología Experimental UAM-I
Lab. Bioenergética y Envejecimiento Celular, Av. San Rafael Atlixco #186, 09340. México.
$norm@xanum.uam.mx

Background: Senescent cells (SC) are characterized by an irreversible cell cycle arrest and by the secretion of several molecules such as cytokines, chemokines, growth factors, and proteases, which altogether are known as senescent associated secretory phenotype (SASP). Physiologically, the SASP promotes an immunogenic program that attracts the adaptive and innate immune systems to clear the SC. However, in pathological conditions, SC are not cleared by the immune system, and their accumulation has been related to tissue dysfunction and tumor progression due to the pro-inflammatory component of the SASP. However, it is still not known if during pathological conditions the SC keep their characteristic immunophenotype that allows them to be detected by the immune system, or if the loss of this characteristic is the cause of their accumulation.

The objective of this work is to identify the markers related to the immunosurveillance of senescent cells, which allow the immune system to recognize and eliminate them.

Methods: Two models of senescence were established in primary mouse fibroblasts. The first one was stress-induced premature senescence (SIPS), which was induced using hydrogen peroxide; the second model was replicative senescence (RS), achieved through exhaustive cell passages in vitro. The senescent state was corroborated by evaluating the expression of cell cycle inhibitors such as p16 and p21, in conjunction with the increased activity of the beta-galactosidase enzyme. To select only SC, the cells were sorted by flow cytometry considering the increase of its fluorescence caused by the cleavage of the C12FDG substrate by the beta-galactosidase enzyme, along with the augment in its size and complexity, characteristics reported in senescent cells. Subsequently, a flow cytometry panel related to activating the elimination of SC by the immune cells such as macrophages and NK was defined, to select the cell population that presents the proposed immunophenotype.

Results: The SC were sorted according to the presence of cycle inhibitors, elevated activity of the beta-galactosidase enzyme, and their increased complexity and size. The chosen immunophenotype was composed of proteins like CD140a, CD47, MCH-I, Ocil, phosphatidylserine, and calreticulin.

Conclusion: These results might allow the generation of new therapeutic possibilities to improve the immune response to eliminate the SC, and therefore prevent diseases related to this process.

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Quantification of gibberellic acid 3 (GA3) in liquid and powder matrix fertilizers by high performance liquid chromatography (HPLC).

Gabriel Hernández Morales, Juan Carlos Doñates Cordero, Luis Manuel Huipe Martínez, José Enrique Botello Alvarez, Marcela Cardenas Manríquez, Ramiro Rico Martínez. Institution: Tecnológico Nacional de México en Celaya, Antonio García Cubas Pte #600 esq. Av. Tecnológico, Celaya, Gto. México. Tel. 01 (461) 61 17575. enrique.botello@itcelaya.edu.mx

Introduction: Gibberellins are bioactive substances that promotes the growth of stems, increase the germination rate of seeds, and induce cell elongation in plants [1]. Extraction methods as well as organic solvents have been evaluated using HPLC.

Objetivs: The general objective is to detect the GA3 in samples available in the market for the validation of the reported concentration.

Methodology: The two powder samples were identified as M1 and M2, M3 and M4 for the two remaining liquid samples. Two treatments were used. In the first treatment (T1), the samples were diluted in mobile phase. In the second treatment (T2) the samples were diluted in HPLC water. Subsequently, three extractions were carried out with ethyl acetate and resuspended in mobile phase.

Results: The detection wavelength for AG3 was 254 nm with a retention time of 3.9 ± 0.102 min. With the recovery percentages and the concentrations obtained from the samples shown in Table 1.1, an estimate was made shown in Table 1.2. The gibberellins present in the samples M3 and M4 don't correspond to the GA3 as show in Figure 1.1.

	T1-%	T2-%	T1	T2
M1	82.4	177.8	1.63	1.46
M2	79.8	16.8	5.14	2.3
M3	55.1	59.0	ND	41.51
M4	79.5	69.6	25.97	25.88

Table 1.1. Percentages of recovery and concentrations of the samples, M1-M2(mg/g) and M3-M4(g/L).

	T1	T2	Product concentration
M1	2.00	0.82	100
M2	6.44	1.42	100
M3	ND	70.36	500
M4	32.65	37.16	40

Table 1.2. Estimated concentrations of samples and products.

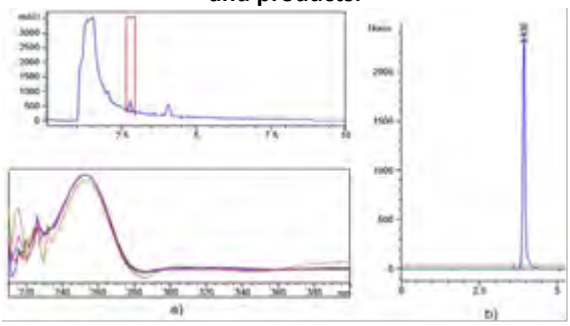

Figure 1.1. Gibberellins with different retention times, a) M3 and b) M4.

The percentage of recovery of the M1-T2 is very high, this was mainly due to the composition of the product. For the M3-T1 the GA wasn't detected.

Conclusions: The GA present in the samples is below the specified concentration of the product. For powder samples it is better dilute and for liquids the solvent washes. Standardizing the analytical methods is of great importance to validate the products.

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Biochemical characterization of recombinant lactate dehydrogenase-1 from white shrimp *Litopenaeus vannamei*

Magally L.E. Hernández Palomares; Elisa M. Valenzuela Soto; Gloria Yepiz Plascencia, Lilia Leyva-Carrillo, Ciria G. Figueroa-Soto
Centro de Investigación en Alimentación y Desarrollo, A.C. Carretera a La Victoria Km 0.6, Hermosillo, Son. Tel: (662) 2892400 ext. 254. elisa@ciad.mx

Under hypoxic conditions, lactate dehydrogenase (LDH, EC 1.1.1.27) plays an important role because it catalyzes the reduction of pyruvate to lactate with the oxidation of NADH to generate NAD+ a limiting substrate for glycolysis. In the shrimp *L. vannamei,* two protein subunits (LDH-1 and LDH-2) were previously identified, deduced from two different transcripts of LDH that come from a unique gene by processing via mutually exclusive alternative splicing. The LDH-2 subunit has been purified and characterized; therefore, the aim of this study was to overexpress and purify the LvLDH-1 recombinant and to study its biochemical characteristics. LvLDH-1 was cloned in the expression vector pTXB1, overexpression was done in *Escherichia coli* ER2566 and purified by affinity chromatography. The native protein was a tetramer with a molecular mass of approximately 140 kDa and higher affinity for pyruvate. The conversion of lactate to pyruvate was almost undetectable which suggests that LDH-1 does not catalyze the reversible reaction. The recombinant LvLDH-1 showed an optimum pH and temperature of 7.5 and 44ºC, respectively. LvLDH-1 was stable at pH and temperature between 8.0 and 9.0 and 20ºC and 60°C, respectively; an activation energy of 2394.7 calories/mol was determined; the enzyme was slightly activated by NaCl and inhibited by ZnCl. In conclusion, the recombinant LvLDH-1 shares some characteristics with LvLDH-2 but it differs in others which explain partially why the LvLDH gene has these two proteins. In addition, LvLDH-1 showed biochemical characteristics similar to the native LDH from other crustaceans, however, there are important differences to these LDHs.

Human Papilloma Virus-18 uses a novel type of mRNA to drive translation of oncoprotein E6 during tumor development

Greco Hernández (1), Alejandra García(1), Giovanna Maldonado (1), José L. González (1), Alejandro García-Carrancá (1,2), Yuri Svitkin (3) and Nahum Sonenberg (3).

From the (1) Division of Basic Research, National Institute of Cancer (INCan), 22 San Fernando Ave., Tlalpan, Mexico City 14080, Mexico; the (2) Department of Molecular Biology and Biotechnology, Biomedical Research Institute, Universidad Nacional Autónoma de México, Mexico City 04510; and (3) the Department of Biochemistry and Goodman Cancer Research Centre, McGill University, 3649 Promenade Sir William Osler, Montreal H3A 1A3, QC, Canada,

Cervix cancer is one of the leading causes of cancer death in women worldwide. The most important etiological cause of this disease is persistent infections with ~~h~~high-risk" (HR) types of human papilloma virus (HPV), mainly types 16, 18, 31, 39 and 45. Viral oncoprotein E6 plays a central role in the carcinogenic properties of HR HPVs in virtue of its interactions with master proteins, such as p53 and c-Myc, causing malfunctioning of several cellular processes that eventually drive tumorigenesis. Nowadays, E6 mRNA translation during cervical tumorigenesis remain poorly understood. We report that in cervical biopsies, in sharp contrast to most eukaryotic mRNAs, HR HPVs transcribe E6/E7 mRNAs with extremely short 5'-UTRs or even lacking it, i.e. 0 — 4 nucleotides-long, to express E6. Using a cell-free *in vitro* translation approach, we prove that E6 ORF translation of HPV-18 mRNAs is driven by a novel motif downstream the AUG start codon.

This motif is conserved in all HPV types of the same genus. Our findings reveal a unique, novel mRNA structure among viruses. We further show that translation of HPV-18 E6 open reading frame largely relays on the cap structure and on translation initiation factors (eIF) 4E and 4AI, two key factors linking translation and cancer development, but does not involve scanning. Our results support the notion that E6 lays at the center of positive oncogenic feedback loops involving E6, eIF4E, mTOR, c-Myc and p53 occurring during tumorigenesis.

Effect of male reproductive tract secreted proteins on human sperm function

Gabriela Hernández-Silva1,3, Jorge Elías Fabián López-Araiza2, Víctor Torres-Flores2, Mayel Chirinos1
1 Departamento de Biología de la Reproducción, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. 2 Facultad de Medicina, UNAM. 3 Posgrado en Ciencias Biológicas, UNAM.
Tel. 5539737531, E-mail gabyhs_1612@hotmail.com

Spermatozoa delivered into the female reproductive tract must undergo physiological changes that enable them to fertilize, a process known as capacitation. Ejaculated sperm plasma membranes are covered by epididymal and accessory glands secreted proteins, but and in order to capacitate and fertilize the oocyte, those proteins must be released from sperm surface. OBJECTIVE: To characterize the peripheral proteins of human sperm released during *in vitro* capacitation and evaluate their effect on sperm function. MATERIAL AND METHODS: Motile sperm from normozoospermic semen samples were obtained by swim up and incubated under capacitating conditions for 6 hours. Proteins released to the medium were recovered and characterized by 2-dimensional electrophoresis and mass spectrometry. Afterwards, the effects of recovered peripheral proteins on sperm protein tyrosine phosphorylation, motility and acrosome reaction were evaluated by Western blot, Computer-Aided Sperm Analyzer and FITC-PSA staining, respectively. RESULTS: Twenty six proteins released by the sperm during *in vitro* capacitation were characterized. Sperm incubation with 1.6 and 3.2 mg/ml of the proteins decreased the protein tyrosine phosphorylation, the percentage of hyperactivated cells and the percentage of sperm with calcium ionophore-induced acrosome reaction. CONCLUSIONS: Proteins released from sperm surface during capacitation have a decapacitating effect with negative consequences for fertilization. The study of each protein on sperm function could contribute to the understanding of their role during capacitation. This research was supported by CONACyT (México).

Proline Content in Commercial Wines

Jesús Abelardo Salazar Alanis [(1)], Mario Alberto Hernández Torres[*(1)], Juan Antonio Rodríguez Arzave [(1)], Wendy Lilian Zenteno Chávez [(1)]

1) Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, Departamento de Química. Av. Pedro de Alba y Manuel L. Barragán s/n, Ciudad Universitaria, San Nicolás de los Garza, Nuevo León, C.P. 66450. Tel: +52 (83) 294000, Ext. 6467, *e-mail: oiramalbert@hotmail.com

Proline is considered physiological indicator of stress in many plants. Typically, proline is the most abundant amino acid in a grapevine juice, the amounts present is influenced by agricultural and genetic factors. During winemaking, the proline content changes little because it is not nutritional importance to yeast. For this reason, knowledge of the proline content can be an indicator helpful for predicting wine quality. Using a spectrophotometric method of acidic ninhydrin in dimethyl sulfoxide, the proline content of nine commercial wine samples were determined. The proline concentration in ranged from 0.54 g/L by California white wine to 1.60 g/L by Chauvenet red wine. White wine samples showed the lowest proline content values, varying from about 0.54-0.58 g/L. These observations were consistent with the proline concentrations previously reported for varietal wines by HPLC analysis. It is not possible to affirm that the wines analyzed showed unfavorable changes during their production. However, reporting the proline content before and during winemaking as well as in the winery could give to the winemaker and wine consumer an additional indicator of quality.

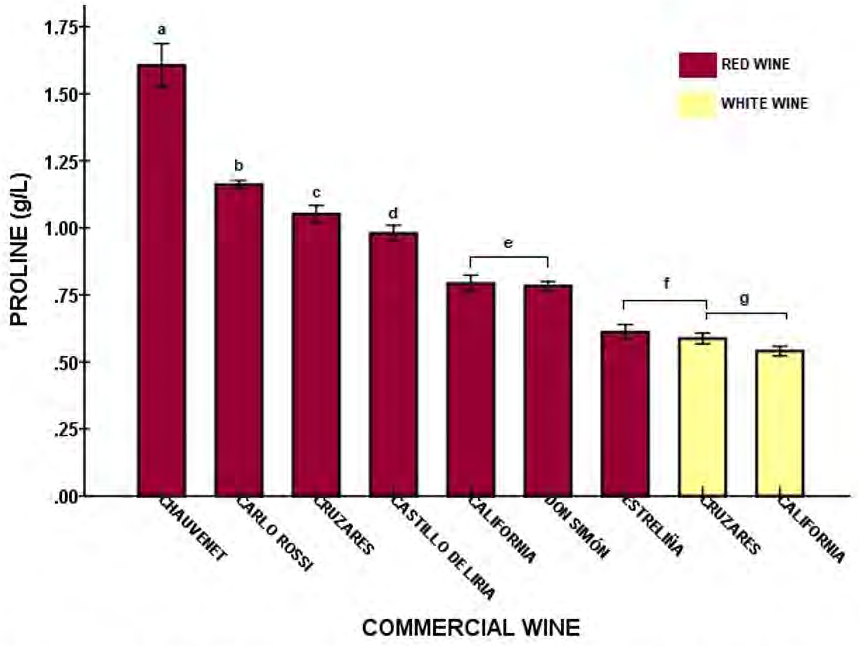

Figure 1. Proline content in commercial wines. Bars with different letters are significantly different (F=914.085, p<0.05).

Proteomics based on LC-MS/MS in tissues of patients chemoresistant to platinum and taxanes with ovarian epithelial cancer.

Gloria Angelina Herrera Quiterio[1] Olga Lilia Garibay Cerdenares[1.2], Berenice Illades Aguiar[1], Sergio Manuel Encarnación Guevara[3], Magdalena Hernández Ortíz[3], Gabriel Martínez Batallar[3], Marco Antonio Jiménez López[4], Mónica Virginia Saavedra Herrera[4], Marco Antonio Leyva Vázquez[1]*.
[1]Molecular Biomedicine Laboratory, Faculty of Chemical-Biological Sciences, Autonomous University of Guerrero
[2]CONACyT- Autonomous University of Guerrero; olgaribayce@conacyt.mx
[3]Proteomics Laboratory, Center of Genomic Sciences-UNAM
[4]Department of Teaching and Research, State Cancerology Institute —DrArturo Beltrán Ortega
*Corresponding author: leyvamarco13@gmail.com Tel.: +52 (747) 4710901
Av. Lázaro Cárdenas S/N Ciudad Universitaria. Chilpancingo, Gro. C.P. 39090 Tel. 017474719310 ext 3601

Carboplatin and paclitaxel are the standard chemotherapy to treat ovarian epithelial cancer (CEO) for more than 30 years. Patients treated with this chemotherapy regimen have a favorable initial response; however, 75% of patients in stages III-IV have resistance to carboplatin within the first 6 and 12 months after the last treatment cycle. The molecular basis for platinum resistance remains largely unknown but is considered multifactorial and numerous mechanisms appear to be involved. In an effort to identify cellular pathways and specific proteins that favor chemoresistance in ovarian neoplasms, this research using a proteomics approach based on liquid chromatography coupled to mass spectrometry (LC-MS/MS) to evaluate the expression of proteins in tissues of patients with CEO chemoresistant to carboplatin compared to cancer-free ovarian tissues (OLC). The proteins were identified by the MAxQuant software, based on their identification code in UNIPROT (www.uniprot.org) and a bioinformatic analysis was performed to detail its function, the possible interaction with other proteins and its role in chemoresistance with the databases: Reactome (http://www.reactome.org/) and GeneOntology (http://geneontology.org/). We identified 3119 proteins, of which 368 are expressed consistently in tissues with CEO and 84 proteins in OLC tissues, among these proteins 54 are expressed in the nine tissues and are associated as cellular metabolism, protein synthesis, cellular transport, cell cycle and immune system, on the other hand 310 proteins are expressed exclusively in tissues CEO these proteins are involved in the activation of diverse biological processes, highlighting the binding to ligands and the catalytic activity of hydrolase and oxidoreductase type, these functions may be related with the process of chemoresistance by preventing antineoplastic drugs from reaching their target site.
These findings indicate the probable role of chemotherapeutics in the differential expression of proteins associated with chemoresistance.

Role of subcellular localization in the subfunctionalization of the paralogous proteins Leu4 and Leu9 in *Saccharomyces cerevisiae*

Jaqueline Hersch González, Alicia González Manjarrez
Instituto de Fisiología Celular / Universidad Nacional Autónoma de México
Circuito Exterior s/n Ciudad Universitaria, Coyoacán, 04510 Ciudad de México
Tel. 55 56225631 / amanjarr@ifc.unam.mx

Gene duplication has been proposed as an adaptive advantage for *Saccharomyces cerevisiae* since selective retention of paralogous genes has resulted in facultative metabolism and the acquisition of the capacity to metabolize both glucose and ethanol as carbon sources. This organism has several pairs of duplicated genes (paralogous) that were generated after allopolyploidization. Duplicated genes go through different evolutionary processes which lead to specialization,subfunctionalization, loss of function or neofunctionalization.

LEU4 and *LEU9* are two paralogous genes which are present in *S. cerevisiae* genome, codifying for two α-isopropylmalate isozymes (α-IPM) that carry out the first step of leucine biosynthesis. These enzymes are localized in the mitochondria and can be organized as heterodimers Leu4-Leu9, or Leu4-Leu4, Leu9-Leu9 homodimers. The three isoforms are differentially regulated by leucine negative feedback, due to leucine inhibition. The Leu9-Leu9 homodimer is highly resistant to leucine inhibition, the Leu4-Leu4 homodimer is hypersensitive, while the heterodimer displays an intermediate sensitivity. Leu4 deletion (*leu4* Δ *LEU9)* results in a lower growth rate as a direct consequence of an increase in *LEU9* expression and intracellular leucine pool, which results in depletion of cells of Citric Acid Cycle intermediates. Lack of Leu9 (*leu9* Δ) has no direct consequence on cell growth rate, whereas a double mutant lacking both *LEU4 and LEU9* is a full leucine auxotroph.

The main purpose of this work is to relocalize Leu4 and Leu9 out of the mitochondria, to the cytoplasm and study the effects of compartmentalization on Leu4 and Leu9 physiological role. We will determine whether Leu4 relocalization to the cytoplasm will have a direct consequence on cell growth rate, on *LEU9* expression and on intracellular leucine concentration; mimicking *leu4* Δ strain phenotype. Furthermore we aim to find if there is heterodimer formation when Leu4 and Leu9 locate in different cell compartments as they do when they are both found in the mitochondria.

Evaluation of the effect of heat capacity on the catalysis of a dimeric enzyme

Ekaterina Jalomo Khayrova, Gloria Saab Rincón*
Instituto de Biotecnología, Universidad Nacional Autónoma de México,
Av. Universidad 2001, Chamilpa, 62210 Cuernavaca, Morelos. +52 (777) 329-1640
* gsaab@ibt.unam.mx.

Enzymatic catalysis has a strong dependence on temperature, the optimum temperature (Topt) is the temperature at which they reach the maximum activity, above this, a decay is observed in the speed of reaction that has been attributed to the denaturation of the enzyme (Tm), at this temperature they lose their three-dimensional structure and consequently, the enzymatic function. Previously the enzymatic behavior with respect to temperature was explained by the Arrhenius and Eyring equation, in this the entropic component was despised because they considered that above the optimal temperature the enzyme began its denaturation process, however, careful analyzes have shown that many enzymes begin their denaturation process some degrees above the optimum temperature. Therefore, the theory of macromolecular velocity (MMRT) has been proposed, in which the dependence of enzymatic catalysis on temperature is explained, with a mathematical rearrangement of the Arrhenius and Eyring equation taking into account the change in the heat capacity and the activation enthalpy and entropy parameters.

The change in heat capacity (ΔCp‡) is a thermodynamic property that describes the difference between the basal state of the enzyme-substrate complex and the enzyme-transition state complex. When this parameter (ΔCp‡) is included as an additional parameter to be defined in a reaction catalyzed by an enzyme, it is found to have a negative value, and it has been experimentally demonstrated that mutations that alter the optimal temperature, modify the value of ΔCp‡. Since the ΔCp‡ of small molecules is not expected to make a significant contribution, the change in ΔCp‡ observed is mainly attributed to the enzyme. The heat capacity of an enzyme, in part, is given by its vibrational modes, which are intimately related to the dynamics of the enzyme.

As a proof of principle, in the present work we propose to modify the vibrational movements of an enzyme without modifying its catalytic site, by increasing its mass. The use of heavy isotopes in half of a homodimeric model will fulfil this requirement allowing to study the catalytic behavior of the unmodified subunit, while inactivating the heavy-one by site-directed mutagenesis to the active-site residues.

The description of the strategy developed is described.

STUDY OF THE TRANSCRIPTIONAL REGULATION OF PARALOGOUS GENES *LEU4* AND *LEU9* IN *Saccharomyces cerevisiae*.

Angélica Mariana Jara Servín, Alicia González Manjarrez
Instituto de Fisiología Celular | Universidad Nacional Autónoma de México
Circuito Exterior s/n Ciudad Universitaria, Coyoacán, 04510 México D.F.
amanjarr@ifc.unam.mx, Tel. +52 55 56225631

Whole Genome Duplication (WGD) in *Saccharomyces cerevisiae* resulted in the retention of an important number of paralogous gene pairs, which conferred various physiological characteristics, depending on whether yeasts are grown on either glucose or ethanol as sole carbon sources. The paralogous genes *LEU4* and *LEU9* codify two isoforms of α-IPMS, which catalize the first reaction of the leucine biosynthetic pathway (acetyl-CoA+ α-KIVa□α-IPM). Leu4 and Leu9 can either form 4/4 or 9/9 homodimers or the 4/9 heterodimer. The formation of the latter is favored since Leu4 and Leu9 are found in a 4:1 proportion, and it has been proposed that Leu4-Leu9 could have highest affinity than that of 4-4 or 9-9. α-IPM's differ in leucine sensibility: 9/9 and 4/4 homodimers, respectively display an (IC50) for leucine of 1.10mM and 0.03mM, respectively, while the heterodimer exhibits an IC50 of 0.16mM. This allows a feedback control, which prevents α-IPM and leucine overproduction through the regulation of α-IPM production, also determining acetyl-CoA flux regulation. Accordingly, simultaneous deletion of both, *LEU4* and *LEU9* genes leads to leucine auxotrophy, while single *LEU4* deletion causes leucine braditrophy, due to formation of the Leu9-leu9 leucine resistant isoform, which leads to intermediate drainage, impairing Krebs Cycly function. Conversely, when *LEU9* is deleted growth is similar to that of the *LEU4 LEU9* wild type strain, since Leu4-Leu4 regulates the system. The transcriptional regulators (TRs) determining *LEU4* and *LEU9* expression have not been yet identified. However, we have obtained results suggesting that Gcn4, Leu3 and Gln3 could be involved in *LEU4* and *LEU9* expression regulation. Furthermore, the fact that in *leu4*Δ mutants, *LEU9* expression is increased, suggested that Leu4 lack could have a metabolic effect resulting in *LEU9* expression through Gcn4, Leu3 or Gln3. Hence, double mutants *leu4*Δ*gcn4*Δ, *leu4*Δ*leu3*Δ and *leu4*Δ*gln3*Δ were constructed and used to evaluate the role of each one of these TRs in *LEU9* expression. Simultaneously, P_{LEU9}*LEU4leu9*Δ and P_{LEU4}*LEU9leu4*Δ chimers were constructed to evaluate the role of *LEU4* and *LEU9* promoters on their expression. Growth kinetics and leucine pools during exponential phase of growth were determined with either glucose or ethanol on pertinent strains. Obtained results suggest that Gcn4 and Gln3 could be involved in Leu4-dependent *LEU9* expression.

NaTrxh, a secreted thioredoxin type *h* from *Nicotiana alata* contains a rare signal peptide possibly recognized only by plant cells

Diana Guadalupe Arzate Peña[1], Andre Zaragoza Gómez[1], Yair Iván Rodríguez Gálvez[1], María Daniela Torres Rodríguez[2], Yuridia Cruz González Zamora[2], Felipe Cruz García[2] y Javier Andrés Juárez Díaz[1*]

[1]. Departamento de Biología Comparada, Facultad de Ciencias, UNAM. Ciudad Universitaria, Cd. Mx. 04510.
[2]. Departamento de Bioquímica, Conjunto E, Facultad de Química, UNAM. Ciudad Universitaria, Cd. Mx. 04510.
[*]Teléfono: (55) 5622 8222 ext. 44750; e-mail: j.a.juarezdiaz@ciencias.unam.mx

Thioredoxins (Trx) are small ubiquitous proteins, widely distributed from prokaryotes to eukaryotes. In plants, the Trx system is quite intricate due to the diversity forms, divided into eight types based on their sequence: *f*, *m*, *x*, *y*, *o*, *z*, *s* and *h*. Trx type *h* (Trx h) form the largest group of this protein family and the information regarding their subcellular localization is limited since there are proteins located in the cytosol, mitochondria and even secreted to the apoplast.

Trx h group is subdivided in three subgroups. Subgroup 2 contains proteins with an Nterminal extension. There are few examples to which this extension has been given an intracellular trafficking role. NaTrxh, a subgroup 2 Trx h from *Nicotiana alata*, has been characterized as a secretion protein in *N. alata* styles. Furthermore, it has been showed that is secreted in *Nicotiana benthamiana* and *Arabidopsis thaliana* leaves and in onion epidermal cells by transient expression assays of the NaTrxh-GFP fusion protein.

From *in silico* analysis of the NaTrxh primary structure, its N-terminal extension is divided in two motifs: Nα (Met-1 to Ala-16) and Nβ (Ala-17 to Pro-27). It has been shown that Nβ leads the secretion of the protein itself in onion epidermal cells. Despite the localization of the Nβ motif within the NaTrxh primary structure and its highly hydrophilic profile, cellular evidences show that the NaTrxh is secreted through the endoplasmic reticulum, the Golgi apparatus and, apparently, is found associated to membrane bodies, presumably secretion vesicles. These data suggest that the recognition/starting point of the NaTrxh towards the secretion RE/Golgi pathway is different from the SRP known route.

Interestingly, when NaTrxh and different N-terminus mutants were expressed in *Saccharomyces cerevisiae*, this protein was presumably always within the cytoplasm,suggesting that this eukaryote cell does not recognize it as a secretion protein, pointing to the possibility that the Nβ motif is a secretion/trafficking information module exclusive for plant cells.

The minimum sequence from the Nβ motif, responsible for the apoplast final localization of the protein that contains it, is being evaluated.

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EFFECT OF IZTLI PEPTIDE 1 ON CELL-CYCLE ARRESTED MAMMALIAN CELLS

Maria Teresa Lara Ortiz, Gabriel Muciño Hernández, Susana Castro Obregón and Gabriel Del Río Guerra. Departamento de Bioquímica y Biología Estructural, Instituto de Fisiología Celular, UNAM. México, D.F. C.P. 04510.
gdelrio@ifc.unam.mx Tel. 56 22 56 63

We have reported a family of antimicrobial peptides derived from α pheromone called Iztli peptides (IP) [1]. The inclusion of α pheromone in these peptides provides them with the ability to induce a cell cycle arrest in *Saccharomyces cerevisiae* cells that express the receptor for the α pheromone (MatA cells). In the initial characterization of one of the peptides, Iztli peptide 1 (IP-1), we show that this maintains an activity similar to pheromone inhibiting the growth of *S. cerevisiae* in MatA cells. More recently we showed that yeast cells must be arrested in G0/G1 for IP-1 to induce cell death; IP-1 may kill yeast cells that are arrested by the activation of the pheromone or any other cellular pathway [2]. The relevance of these findings is because IP-1 is the first antibiotic reported to selectively kill arrested cells, while all previously known antibiotics are not toxic against cell-cycle arrested cells. In this regard, we have previously shown that IP-1 is not toxic to actively dividing mammalian cells [3]. Hence, the aim of this study is to determine if IP-1 may induce cell death during a cell cycle arrest through a conserved mechanism in eukaryotes. The first evidence we observed about the conservation of this mechanism is the ability of IP-1 to bind to homologous proteins in yeast and human cells. Considering the conservation of the cell cycle mechanism between yeast and mammals, we are now studying the toxic activity of IP-1 in primary cultures of MEF (Mouse Embrionic Fibroblasts) during different forms of cell cycle arrest, including arrest induced by confluence or by chemical compounds. The results of these experiments will be presented during the meeting.

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EFFECT OF MODERATE EXERCISE AND METFORMIN TREATMENT ON MITOCHONDRIA ISOLATED FROM OLD RATS QUADRICEPS.

Stefanie Paola López-Cervantes[1], Norma Silvia Sánchez-Sánchez[2], Martha Calahorra-Fuentes[2], David Hernández-Álvarez[1], Antonio Peña-Díaz[2], Mina Konigsberg-Fainstein[1]
[1]Laboratorio de Bioenergética y Envejecimiento Celular, Departamento de Ciencias de la Salud. Universidad Autónoma Metropolitana, Unidad Iztapalapa. San Rafael Atlixco 186, Col. Vicentina, Iztapalapa, 09340, Tel. 58044727 mkf@xanum.uam.mx
[2]Departamento de Genética Molecular, Instituto de Fisiología Celular, UNAM.

Aging is a natural and inevitable process that is characterized by a progressive physical and mental deterioration, leading to an accumulation of dysfunctional components, and ending with death. In the skeletal muscle, the progressive mass and strength loss is known as sarcopenia. The etiology of sarcopenia has been related to factors such as oxidative stress, protein homeostasis alteration, apoptosis, inflammation and mitochondrial dysfunction. Particularly, mitochondria play an important role in muscle deterioration related to aging due to their importance in energy production, as well as in relation with reactive oxygen species generation and apoptosis signaling. It has been suggested that exercise might produce beneficial effects in the mitochondrial life cycle, which has effects on the main signaling pathways, which control the quality and quantity of mitochondria during aging. The contraction of skeletal muscle during physical exercise activates the mitochondrial response and biogenesis, as well as mitochondrial replacement by mitophagy. Metformin (MTF), a biguadine that has been used as a type 2 diabetes treatment for more than 60 years, is known to improve physical performance, recover insulin sensitivity, and decrease cholesterol and low density lipoproteins. At the molecular level, MTF increases AMPK activity and antioxidant response. It has been proposed that MTF inhibits complex I activity in the mitochondrial electron transport chain, and therefore activating AMPK. So our question was to understand whether the MTF treatment + moderate exercise could avoid the mitochondrial dysfunction in old rats. So female Wistar rats were treated with MTF (100 mg / kg) daily for 12 or 6 months along with a moderate exercise routine (25 cm / s, 30 minutes a day, 5 days a week) which started at 4 or 12 months of age. Mitochondria were isolated from quadriceps skeletal muscle at 24 months of age and mitochondrial respiration was determined with a Clark electrode. Mitochondria obtained from animals treated with MTF + exercise had a better performance compared to animals that only executed exercise or were sedentary.

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Single residue change causes loss in phospholipase and hemolytic activity on *Vibrio paraheamolyticus* thermolabile hemolysin

Alonso Alexis López-Zavala [a,*], Michelle Adriana Grajeda-Gutierrez [a], Luis Enrique Vazquez-Morado [a], Adrián Ochoa-Leyva [b] and Filiberto Sánchez[b].

[a]Laboratorio de Investigación en Alimentos. Universidad de Sonora. [b]Departamento de Microbiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México. *corresponding author: alexis.lopez@unison.com, Hermosillo, Sonora, C.P. 83000, México. Tel. (662)172 2240.

Vibrio parahaemolyticus (*Vp*) is a bacteria associated to infection processes as early mortality syndrome and systemic vibriosis in shrimp aquaculture. Infectious *Vp* strains for shrimp are positive for lecithin dependent hemolysin (LDH) but could be negative for human pathogenic markers as thermo-direct (*tdh*) and thermo-related (*trh*) hemolysins. *Vp* uses both *thd* and *trh* pore forming hemolysin as part of the infection mechanisms. Meanwhile, *Vp*LDH is designated as an atypical phospholipase B/A_2 and lysates sheep erythrocytes. Amino acid sequence analysis indicates a conserved catalytic motif belonging to GDSL esterase family. A conserved catalytic serine in position 153 could be responsible of both hemolytic and enzymatic activity. We characterize the effect of a single mutant (S153G) of *Vp*LDH. S153G_*Vp*LDH was overexpressed as a His-tagged protein in *E. coli* as inclusion bodies, purified under denatured conditions with urea 8 M by nickel-affinity chromatography. 12% SDS-PAGE confirmed the presence of a single band ≈ 47kDa. The purified protein was refolded by dialysis in several steps eliminating urea from 8 to 0 M. No clear/opaque-concentric circles were observed when renatured S153G_*Vp*LDH was assayed using a qualitative egg-yolk phosphatidylcholine in agar-plates assay, indicating loss both B and A_2 in phospholipase activity. Also, only 7 % of residual esterase activity was detected with p-nitrophenyl laurate as substrate compared to wild type *Vp*LDH. In other hand, S153G_*Vp*LDH was not able to lysate human erythrocytes. This result indicates that serine plays an important role in *Vp*LDH functionally and could be a target for inhibition.

Switch expression of MATs proteins in a sequential model of cirrhosis-hepatocellular carcinoma induced by DEN and the hepatoprotective effects of IFC305.

María Guadalupe Lozano-Rosas, Gabriela Velasco-Loyden, Mariana Domínguez López, Enrique Chávez and Victoria Chagoya Hazas. Instituto de Fisiología Celular, UNAM, Del. Coyoacán, 04510, Cd.Mx. México. Tel: +52(55)5622-5614. Fax: 56225611. E-mail: vchagoya@correo.ifc.unam.mx

Cirrhosis and Hepatocellular carcinoma (HCC) are complex processes that involve a dynamic modification of liver cells phenotype; their pathological characteristics are the generation of fibrosis that results on structural distortion and dysfunctional liver. Transcriptome assays made in our laboratory showed that 414 differential genes are deregulated in liver samples, tended to be normalized by an adenosine derivative compound, IFC305 (UNAM Patent 207422). Some of these genes were the MAT enzymes, *Mat1a* and *Mat2a*, which are expressed in the health liver and extrahepatic tissues, respectively. S-adenosylmethionine (SAM) is the biological methyl donor and its synthesis is catalyzed by methionine adenosyltransferase (MAT). In HCC, there are a MAT protein switch expression; favoring MAT2A, affecting the level of SAM, and depleting the level of MAT1A. In this work we evaluated if the hepatoprotector IFC305 (an adenosine aspartate derivative) has the ability to modulate the switch expression of MATs and the synthesis of SAM in an experimental model of cirrhosis-HCC. Male Wistar rats were treated for 12 weeks with DEN for cirrhosis and 16 weeks for HCC induction, and two different groups were simultaneously treated with IFC305. As previously mentioned, the diminution of MAT1A expression and the increased in MAT2A were recuperated to the normal values after the treatment with the IFC305 compound.

Thus, these results are part of the beginning in the comprehension of the metabolic deregulation in the DEN induced model and the possible hepatoprotective mechanism of the IFC305.

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Contribution of the Mitochondrial Intrinsic Pathway to the Apoptosis Resistance exhibited by Fibroblasts from Human Idiopathic Pulmonary Fibrosis

E. R. Luis-García1, C. Becerril1, J. Pedraza-Chaverri2, M. Orozco-Ibarra3, E. Aparicio-Trejo2, C. Mendoza-Milla1, A. Salgado-Aguayo1, A. Pardo4, M. Selman1.
1Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Mexico, Mexico, 2Facultad de Química, Universidad Nacional Autónoma de México, Mexico, Mexico, 3Instituto Nacional de Neurología y Neurocirugía, Mexico, Mexico, 4Facultad de Ciencias, Universidad Nacional Autónoma de México, Mexico, Mexico.

Idiopathic Pulmonary Fibrosis (IPF) is a chronic and frequently lethal disease of unknown etiology, characterized by the excessive accumulation of extracellular matrix produced by fibroblasts. Previous reports have shown that IPF fibroblasts are resistant to Fas-mediated apoptosis (extrinsic pathway); however, it is not known whether the apoptotic intrinsic athway is also involved in this resistance. The inhibition of the mitochondrial permeability transition pore (mPTP) has been proposed as a mechanism related to resistance to apoptosis. Then, our aim is to analyze the role of the mitochondria on the apoptosis resistance of fibroblasts from IPF and whether the inhibition of the mPTP contributes to it.

Human fibroblasts isolated from pulmonary biopsies of two adults without pulmonary diseases (Control) and two IPF patients matched by age were used. Control and IPF fibroblasts were studied under basal conditions and after mitomycin C-induced apoptosis. Early apoptosis was analyzed by flow cytometry with Annexin V/IP, cytochrome c release in the cytosolic fraction was analyzed by HPLC and Western blot and mPTP opening was assessed by the quenching of calcein-AM fluorescence by cobalt chloride through flow cytometry and confocal microscopy.Moreover, mitochondrial respiration was measured as the oxygen consumption in permeabilized and non-permeabilized cells using a type Clark electrode or an Oroboros Instrument respectively.

We found that IPF fibroblasts are resistant to mitomycin C-induced apoptosis and showed less cytochrome c release than control fibroblasts. Studies of co-localization between calcein-AM and cobalt chloride suggested a closer mPTP in IPF fibroblasts. Also, the mPTP of IPF fibroblasts was found less sensitive to ionomicin-induced opening. Regarding oxygen consumption, IPF fibroblasts showed decreased: a) maximum respiratory velocity, b) respiratory control, and c) basal respiration compared to control fibroblasts. Furthermore, tests with permeabilized cells suggested a decrease in complex I function. In addition, preliminary experiments showed that mitochondrial potential is decreased in IPF fibroblasts. We will evaluate if the phenotype observed in IPF fibroblasts can be reverted by compounds that favor OXPHOS such as methylene blue.

In conclusion, our results support the mPTP contribution to the apoptosis resistance exhibited by IPF fibroblasts, and this event is related with failure in mitochondrial respiration.

CETPI Derived Peptide Improves Acute Phase Response in a Septic Shock *in vivo* Model

Ismael Luna Reyes1, Erendira Perez Hernandez1, Jaime Mas Oliva*1, Alejandro Zentella Dehesa2, Miguel Ángel Ávila Rodriguez3
1. Departamento de Bioquímica y Biología Estructural, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México.
2. Unidad de Bioquímica Dr. Guillermo Soberón Acevedo, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán
3. Unidad PET-CT, Facultad de Medicina, Universidad Nacional Autónoma de México.
*Corresponding: Laboratorio 322 Norte, Departamento de Bioquímica y Biología Estructural, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México. Cto. Exterior S/N, Ciudad Universitaria, 04510, Ciudad de México, CDMX.
Tel. +52 55 (5255) 56225584
e-mail: jmas@ifc.unam.mx

Cholesterol Esther Transfer Protein Isoform (CETPI) is a protein that was originally described in our laboratory, CETPI only differs from CETP (Cholesterol Esther Transfer Protein) in the last eighteen amino acids localized in the C-terminal región, due to this fact CETPI loses its cholesterol transfer properties(1). These last eighteen amino acids from CETPI were used to build a peptide, VSAK peptide, that has demonstrated Lipopolysaccharide (LPS) binding properties and neutralizing effects in both *in vitro* and *in vivo* models(2).

In our present study, we analyzed the effects of administration of VSAK peptide in a septic shock model. We implemented a septic shock model based in the intravenous administration of LPS in dwarf Dutch male rabbits, ten rabbits were randomly assigned for the administration of LPS, VSAK or both (VSAK+LPS); a saline group was used as a control. We assessed metabolic changes through Positron Emission Tomography (PET) using 18-Fluorodeoxyglucose as a radiotracer. Changes in cytokine profile, both pro-inflammatory and anti-inflammatory, were also measured using a multiplex assay.

In accordance with our previous work, VSAK peptide shows a beneficial effect when is administered in rabbits than also were administered with LPS. Data from PET assays shows than rabbits that were administered with LPS alone showed a major decrease in Standard Uptake Value (SUV) than those that were administered with both LPS and VSAK, non-important changes in SUV were observed between Control, VSAK+LPS or VSAK-alone groups.

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Search of small organic molecules that modulate the function of the GTPase EFL1

Miryam Samantha Maldonado López, Nuria Victoria Sánchez Puig, Karina Martínez Mayorga
Instituto de Química, Universidad Nacional Autónoma de México
Circuito Exterior s/n, Ciudad Universitaria. Delegación Coyoacán, C.P. 04510, México, D.F. Tel. +52 (55) 56 22 44 68 nuriasp@unam.mx

The Shwachman-Diamond syndrome (SDS) is an autosomal recessive disorder characterized by exocrine pancreatic insufficiency, bone marrow dysfunction, skeletal abnormalities and an increased risk of leukemia. SDS is the second most common cause of exocrine pancreatic insufficiency in children. Approximately 90% of SDS patients have biallelic mutations in the SBDS gene. Studies with the yeast orthologue suggest SBDS protein may act as a guanine nucleotide exchange factor for EFL1. These proteins catalyze eviction of the antiassociation factor (eIF6) from the surface of the 60S subunit. Recently in our research group, have been identified mutations in EFL1 from SDS patients. These, same as the mutations in SBDS, avoids the eIF6 release and the recycling to the nucleus. eIF6 sterically blocks ribosomal subunit joining, its release depends of a conformational change in EFL1. EFL1 is homologous to the ribosomal translocase elongation factor 2 (EF2), it has been proposed that the translocation is similar to that EF2 undergoes during protein synthesis. Sordarin is a toxin capable of emulating the pre-translocated state on EF2 by binding in an interdomain region. We consider that if the conformational change that EFL1 undergoes to remove eIF6 is similar to the pre-translocated state generated by sordarina in EF2, then a small organic molecule could emulate the translocated conformation on EFL1 by coupling to the equivalent sordarin binding site. Proposing EFL1 as a therapeutic target of SDS. We have performed similarity and docking studies on EFL1 with a commercial compound library and we have identified candidate molecules that could interact with the GTPase EFL1.

Application of a biosensor and computational tools for studies of molecular interaction of some drugs related to calmodulin.

Deyamira Matuz-Mares, Isabel Velázquez-López, Georgina Garza-Ramos, Juan Pablo Pardo, Rogelio Rodríguez-Sotres, and Martín González-Andrade. C.P. 04510, Tel: 56232254, Fax: 56162419; martin@bq.unam.mx. Department of Biochemistry, Faculty of Medicine, UNAM, Av. Universidad 3000.

Abstract

In this work, we studied the interactions at the molecular level of five drugs and two endogenous metabolites with the Calmodulin protein (CaM), using a CaM biosensor, docking and molecular dynamics. Experimental results showed *K*d ranging from 0.71 to 6.7 μM; and for serotonin (**5-HT**) whose title and *K*d is reported for the first time, thanks to the sensitivity of the *h*CaM M124C-*mBBr* biosensor. The affinity of the ligands shows the following binding, using the biosensor: **5-HT** > vincristine (**VCT**) > chlorpromazine (**CPZ**) > fluoxetine (**FLU**) > vinblastine (**VBT**) > imipramine (**IMI**). The theoretical results show a good correlation with the experimental results. The docking studies indicate that CaM has at least two probable binding sites depending on the ligand. The molecular dynamic (MD) studies of all complexes were made by 100 ns. The analyses of the MD trajectories give us theoretical thermodynamic parameters. The enthalpic component contributes in greater proportion to the ΔG in Ca2+-CaM-Drug complexes, except for Ca2+-CaM-Dopamine. Studies of the different compounds both experimentally and theoretically have different degrees of affinity for CaM and this may be related to pharmacological action. Therefore, our results show the importance of bioanalytic devices with greater sensitivity, as well as computational tools that complement the experimental information.

SLC16A11: A transporter associated with the risk of Type 2 Diabetes in Mexicans

Andrea Celeste Medina García, José Luis Ventura Gallegos, Juan Carlos León Contreras, Saúl Cano Colín, Braulio Martínez Benítez, Arturo Ángeles Ángeles, Teresa Tusié Luna.
Unidad de Biología Molecular y Medicina Genómica del IIB-UNAM/INCMNSZ,
Vasco de Quiroga No. 15, Col. Belisario Dominguez Sección XVI, Tlalpan C.P. 14080. 5687-0900, ext. 2252. andreamedina@ciencias.unam.mx

Through genome-wide association studies (GWAS), an haplotype on the *SLC16A11* gene located at chromosome 17p13, was found as the major risk factor for type 2 diabetes (T2D) in Mexicans. Each risk allele, increased disease risk by 25%, and this *locus* explains 20% of the increased T2D prevalence in Mexico. The T2D-risk haplotype includes five coding variants in the *SLC16A11* gene, comprising four missense and one synonymous mutations. A recent publication showed that the variants in the T2D-associated haplotype have two distinct effects, one, decreasing the expression of *SLC16A11* in liver and disrupting a key interaction with Basigin (BSG), a chaperone protein important for localization of the transporter at the plasma-membrane, thereby reducing cell-surface localization.

SLC16A11 is mainly expressed in thyroid, liver and salivary gland and the protein encoded by this gene is a member of the monocarboxylate transporter (MCT) family. These proteins (category I) transport simple monocarboxylic acids such as lactate, pyruvate and ketone bodies, via a proton (H+)-coupled mechanism and interact with BSG. There is a second class of transporters (category II), these proteins transport larger hydrophobic monocarboxylates such as triiodothyronine (T3), thyroxine (T4) and aromatic amino acids through facilitated diffusion and do not interact with BSG; nevertheless *SLC16A11* has not been studied and its functions and role in the metabolism is largely unknown. A recent study showed that SLC16A11 can marginally transport pyruvate (despite the presence of a major MCT1 transporter in the plasma membrane of human hepatocytes), and the presence of the 5 amino acids of the risk haplotype in *SLC16A11*, induces changes in fatty acids and lipid metabolism associated with increased T2D risk.

We have built a structural model of *SLC16A11* to be evaluated by molecular docking with diverse ligands. Our findings suggest that there is a more favorable interaction with T3, T4 and aromatic amino acids. Also, we constructed a phylogenetic tree of the MCT family and found that *SLC16A11* is closely related with the members of the MCT II class. Our experiments *in vivo* with HepG2 and 293T cell lines as well as with human liver samples suggest the existence of protein isoforms. Currently, we are working on analyzing the subcellular distribution of the protein using differential centrifugation and immuno-localization with colloidal gold. Preliminary results show its presence in mitochondria, plasma-membrane and nucleus. Is therefore possible that different isoforms can be found at different tissues and cellular compartments and each one might have differential substrate specificity.

Thermodynamic and kinetic characterization of the unfolding of the B domain of the Lysine-arginine-ornithine binding protein (LAO). Comparison with the behavior of the A domain and the wild type protein.

Eva Isela Mejía Juárez, Tania Berrocal Gama, Jesús Renán Vergara Gutiérrez,

Alejandro Sosa Peinado, Daniel Alejandro Fernández Velasco. Laboratorio de Fisicoquímica e Ingeniería de proteínas, Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad Universitaria. Ciudad Universitaria, 04510, Ciudad de México, México. Phone number +52 5574762829. E-mail: evaisela2012@gmail.com (EIMJ), fdaniel@unam.mx (DAFV)

The information required for proteins to get their tridimensional structure, is contained on its amino acids sequence. However it is not possible yet to determinate the tertiary structure from its primary structure. Most of the studies of folding have been conducted on model small proteins (<100 residues) composed of only one domain, however most of the protein in the cell are more complex. For this reason, we have previously conducted a kinetic and thermodynamic study of the periplasmic lysine-arginine-ornithine binding protein (LAO), a bilobular protein of 238 residues made of a continuous domain (A domain), and a discontinuous one (B domain). This work has shown that LAO presents a two-state folding process (ΔG = 9.8 Kcal mol-1 at pH 9.0) and a kinetic folding mechanism characterized by parallel pathways with an in pathway intermediate. With the purpose of discovering if this characteristics arise in an additive way by the independent folding of each of the LAO domains, or whether they are a consequence of its interaction, the kinetic and thermodynamic properties of the isolated A and B domains are being studied. Thermodynamic data showed that despite of being discontinuous, the A domain folds properly, showing two state behavior, with a ΔG of 5.5 Kcal mol-1. This value being proportional to the number of residues that compose the domain). Kinetic experiments showed that the folding mechanism of the A domain is more complex than that of the whole LAO protein, contrary to what was expected. On the other side, the B domain presents a marginal stability at pH 9.0 (ΔG = 0.8 kcal mol-1), which is lightly increased at pH 5.0 (ΔG = 1.4 Kcal mol-1). Interestingly, kinetic studies of the B domain indicate that its folding rate is higher than those of the LAO and A domain. It also shows the presence of an off pathway intermediate. Finally, we can conclude that, despite being discontinuous, the A domain of LAO folds properly. While the B domain needs the other domain in order to be stable, suggesting a cooperative behaviour. As proposed by other authors, we found that the folding rate depends on the topological complexity and size of the protein. We found however, that folding complexity does not depend on these properties, rather,folding is more complex in the independent domains than in the whole wild-type protein.

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Buthionine sulfoximine is a multi-target inhibitor of trypanothione synthesis in Trypanosoma cruzi.

Marlen Mejia Tlachi, Citlali Vázquez Martínez, Zabdi González Chávez, Jose Salud Rodríguez-Zavala, Rafael Moreno-Sánchez, Emma Saavedra
Departamento de Bioquímica, Instituto Nacional de Cardiología Ignacio Chávez; Juan Badiano No. 1 Col. Sección XVI, Tlalpan, Ciudad de México, 14080, México, Tel. (+5255) 5573-2911 ext. 25500. email:emma_saavedra@hotmail.com

The antioxidant metabolism of Trypanosoma cruzi depends on trypanothione [T(SH)2], a conjugate of two glutathione (GSH) and a spermidine; T(SH)2 is the functional analog of GSH in mammalian cells.
Buthionine sulfoximine (BSO) is an inhibitor of γECS, the first enzyme in the GSH synthesis pathway. BSO induced 60-80% decrease of the intracellular GSH and T(SH)2(1) in T. cruzi. Another study in Trypanosoma brucei(2) showed 80% decreased GSH and T(SH)2 pools in parasites treated with BSO,an effect that was not prevented with GSH supplementation. These results suggested that BSO has additional targets to γECS. We propose that the decrease of T(SH)2 in parasites treated with BSO and supplemented with GSH is due to inhibition of trypanothione synthetase (TryS), the enzyme responsable of T(SH)2 synthesis. The objective of this work is to determine if BSO is a nonspecific inhibitor of T(SH)2 synthesis.
The IC50 of BSO on T. cruzi epimastigotes growth was 4 mM. Parasites treated with BSO, showed 50% cysteine (Cys) accumulation, 50% decrease in GSH and T(SH)2 depletion. Subsequently,experiments of BSO inhibition were carried out in the absence and presence of extracellular Cys or GSH to circumvent Cys defficiency for □ECS and GSH deficiency for TryS, respectively. Both thiols were internalized by the parasites, increasing the T(SH)2 pool 4-7 folds. However, by supplementing the parasites with Cys or GSH in the presence of BSO, the cells did not synthesize more T(SH)2 despite of having 80-120% internal GSH content. These data strongly suggested that TryS was inhibited by BSO. To demonstrate this, T. cruzi epimastigotes overexpressing γECS (oe-γECS) and TryS (oe-TryS) showed 63% and 55% decreases in the overexpressed activities, respectively.
Furthermore, γECS, and TryS, but not glutathione synthetase, recombinant enzymes were inhibited with D,L-(S,R) –BSO. For TryS, BSO was a competitive inhibitor against GSH with a Ki value of 170 μM. These results showed that BSO is an inhibitor of γECS and TryS.
Benznidazole (Bnz) is the preferred antichagasic drug despite its low efficacy and high toxicity; hence a combination therapy to eliminate the T. cruzi infection could be advantageous. An IC50 of 11 μM of Bnz was determined on T. cruzi growth. The combination of Bnz plus BSO decreased the IC_{50} value to 5 μM.
We can conclude that BSO is not an specific inhibitor, in addition to inhibiting γECS, it also inhibits TryS, which as a whole explains the drastic decrease of the T(SH)2 pool in the parasite. Furthermore,a combination therapy using low doses of Bnz and BSO can be proposed to kill the parasites.

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Structure-based identification of a potential non-catalytic binding site for rational drug design in the fructose-1,6-biphosphate aldolase from *Giardia lamblia*

Sara Teresa Méndez, Adriana Castillo-Villanueva, Horacio Reyes Vivas, Jesús Oria-Hernández.
Laboratorio de Bioquímica-Genética, Instituto Nacional de Pediatría, Instituto Nacional de Pediatría. Insurgentes Sur 3700-C, Col. Insurgentes Cuicuilco, Delegación Coyoacán, Ciudad de México, CP 04530, México. Tel: +52 55 1084 0900 Ext 1442; E-mail: jesus.oria.inp@gmail.com.

Giardia lamblia is the causal agent of giardiasis, one of the most prevalent parasitosis in the world. Even though effective pharmacotherapies against this parasite are available, the problems associated with its use (drug resistance, for example) indicate that novel antigiardiasis options are needed. Based on its dependence on glycolysis as main source of ATP, glycolityc enzymes have been proposed as plausible targets to design enzyme inhibitors with antigiardial potential. Among this, fructose-1,6-biphosphate aldolase (GlFBPA) has been highlighted as a promissory target for novel antigiardial drug design. Current efforts are based on design competitive inhibitors of GlFBPA; however, in the kinetic context of metabolic pathways, competitive inhibitors seem to have low real potential as therapeutic agents. In this work we performed an *in-silico* structure-based approach to discover a non-catalytic binding site region which could be used as molecular target for subsequent drug design. The druggability of the selected region was experimentally tested; the alteration of this region by site directed mutagenesis disturbs the catalytic properties and the stability of the enzyme. The results validate the proposed region as a plausible molecular target with pharmacological potential.

Study of the putative nuclear localization signals (NLS) of the cationic channel TRPV4

Susana Méndez-Gómez, Heidi Gabriela Espadas-Álvarez, Ivette Rodríguez-Ramírez, Refugio García-Villegas.
Departamento de Fisiología, Biofísica y Neurociencias1, CINVESTAV-Zacatenco. Av. Instituto Politécnico Nacional 2508, San Pedro Zacatenco, 07360 Ciudad de México. Tel. 57473800, Ext. 5102. mendezgs@fisio.cinvestav.mx

Transient receptor potential vanilloid 4 (TRPV4) is a non-selective Ca2+ permeable channel activated by different stimuli, including hypoosmolarity, warm temperature,mechanical forces and endogenous metabolites such as arachidonic acid. It has also, -specific activators such as 4α-PDD and GSK1016790A and specific-inhibitor RN1734. TRPV4 is a 871 aminoacids protein with six transmembrane regions and intracellular amino- and carboxy-terminal domains. Functional channels are tetramers but it can also form heterotretramers with TRPC1 and TRPP2 channels,this last also known as PKD2, a protein involved in polycystic kidney disease.

Since TRPV4 is expressed mostly in the kidney, we used the renal epithelial MDCK cell line as a model to study the TRPV4 channel function in renal physiology. In our workgroup, we have found that in sparse cultures of MDCK cells, functional TRPV4 channels are localized in the cellular nucleus where they regulate the transcriptional activity of β-catenin protein. Depending on TRPV4 channel activation state its subcellular localization changes and is shuttling between the nuclear and the cytoplasmic regions. *In silico* analysis of the TRPV4 sequence identified along this protein at least four putative nuclear localization signals (NLS). We are evaluating the functionality of each of them by site-directed mutagenesis and subsequent analysis by immunofluorescence and confocal microscopy.

We also explored the hypothesis that a phosphorylation site in S824 (near of a putative NLS in the COOH domain) could affect the nuclear traffic of the TRPV4 channel. We generated two S824 mutants, one phosphoresistant and one phosphomimetic: TRPV4-Flag-S824A and TRPV4-Flag-S824D respectively. We observed after 72 h of transfection, that the while wild type and phosphoresistant construct present cytoplasmic and plasma-membrane location, the phosphomimetic construct remains in the nucleus. This result suggests that the phosphorylation state of TRPV4 channel affects its permanence in the nucleus.

Identification of microRNAs related to obesity in Mexican children.

Carolina Miranda-Brito2,3, Diana Lucía Mendoza-Monzoy3, Marta Romano-Pardo2, Miguel Cruz López1, Jaime García-Mena3. 1Unidad de Investigación Médica en Bioquímica, CMN SXXI. 2Departamento de Fisiología, Biofísica y Neurociencias, 3Departamento de Genética y Biología Molecular. Cinvestav Unidad Zacatenco. Av. Instituto Politécnico Nacional 2508, Col San Pedro Zacatenco, CDMX 07360, México. (55) 5747-3800 X5328. jgmena@cinvestav.mx

The prevalence of obesity in the World and the Mexican population has increased alarmingly. Currently in our country, over 26% of children present an overweight problem or obesity, which represents a serious public health problem. Obesity is a multifactorial metabolic disease associated with the risk of developing chronic diseases. It is important to analyze the factors that influence the expression of genes related to the metabolic processes.

MicroRNAs (miRNAs) are a class of small RNAs, endogenous and non-coding, which repress the translation of mRNAs involved in important cellular processes. Alterations in the expression and function of miRNAs had been linked to pathophysiological states. Circulating miRNAs are found in several fluids and are associated with lipoproteins and micro-vesicles of different cell types suggesting them as a type of intercellular communication. It is reported that there are characteristic patterns of miRNAs in plasma of patients with cancer and diabetes, which may allow us to use them as noninvasive biomarkers.

In this study we measured the levels of six miRNAs previously associated with the development of obesity (miR21, miR33, miR103, miR132, miR143 and miR221) in plasma samples of Mexican children aged 6 to 12 years-old, under a case-control study, miRNA concentration was measured using qPCR- SYBR® Green. Children were selected and classified based on body mass index reported in percentiles.

It was observed that miR21 and miR132 are present in lesser concentrations in the plasma of obese children. Furthermore, the miR-33 was found at higher levels in the case group over the control. From the above it is concluded that differences in the levels of certain microRNAs in plasma that allow associate with the presence of obesity, this knowledge helps supplement the overview of the etiology of this condition.

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Silencing of HIF-1 decrease the viral load and restore the antioxidant response in WSSV-infected white shrimp

Melissa María Miranda-Cruz1, Jennifer Joceline Poom-Llamas1, Denisse Verduzco-Ávila1, Diana Lourdes Padilla-Bórquez1, Susana Castro-Meza1, José Arquímides Godoy-Lugo2, Rudy Ortiz2, Jesús Alfredo Rosas-Rodríguez1, José Guadalupe Soñanez-Organis1*.
1Universidad de Sonora, Depto. de Ciencias Químico-Biológicas y Agropecuarias, Universidad de Sonora Unidad Regional Sur, Apartado Postal 85880, Navojoa, Sonora, México.
2School of Natural Sciences, University of California Merced, 5200 N Lake Road, Merced, CA 95343, USA.
*Corresponding author: Dr. José Guadalupe Soñanez-Organis Depto. de Ciencias Químico Biológicas y Agropecuarias Universidad de Sonora Unidad Regional Sur Apartado Postal 85880, Navojoa, Sonora, México Email: jorganis@navojoa.uson.mx or josegpeso@gmail.com

ABSTRACT

Hypoxia inducible factor -1 (HIF-1) is transcriptional factor that induces genes involved in glucose metabolism. HIF-1 is formed by a regulatory α-subunit (HIF-1α) and a constitutive β-subunit (HIF-1β). The white spot syndrome virus (WSSV) induces shift in glucose metabolism shift and oxidative stress production. HIF-1α is associated with the induction of metabolic changes in tissues of WSSV-infected shrimp. However, the contribution of HIF-1 to viral load and antioxidant responses in WSSV-infected shrimp have been not examined. The viral load increased in hepatopancreas and muscle after WSSV infection, and the accumulative mortality was of 100% at 72 h post-infection. The expression and activity of SOD, catalase, and GST decreased in each tissue evaluated after WSSV infection. Protein carbonyl concentrations increased in each tissue after WSSV infection, while lipid peroxidation increased in hepatopancreas, but not in muscle. Silencing of HIF-1α decreased the WSSV viral load in hepatopancreas and muscle of infected shrimp along with shrimp mortality. Silencing of HIF-1α ameliorated the antioxidant response in a tissue-specific manner, which translated to a decrease in oxidative damage. These results suggest that HIF-1 is essential for successful viral replication and restores the antioxidant response, which counters the oxidative injury associated with WSSV infection.

ANTIFUNGAL AND ANTIOXIDANT ACTIVITY OF HYDROLYSATES FROM MELÓN SEED STORAGE PROTEINS (*Cucumis melo L.*)

Francisco Xavier Mojica Rodriguez1; Irma Bernal Lugo2; Juan Gabriel Ramírez Pimentel1; César Leobardo Aguirre Mancilla1; Gabriel Iturriaga de la Fuente1; Juan Carlos Raya Pérez1
1Tecnológico Nacional de México. Instituto Tecnológico de Roque. km 8 carretera Celaya-Juventino Rosas. C.P. 38110. Celaya, Gto., México; 2Universidad Nacional Autónoma de México. Facultad de Química, Circuito Exterior S/N, Coyoacán, Cd. Universitaria, 04510 Ciudad de México, CDMX
Autor para correspondencia: irmofel@unam.mx

Introduction. Plants has developed protection mechanisms against phytopathogens, in which, peptides with antimicrobial activity (AMPs), play an important role. In seeds, these peptides are inactive in the sequences of the storage proteins. The enzymatic hydrolysis seems to be the most effective and preferred method to produce them. The proteases used and hydrolysis conditions, will determine the composition of these peptides, their net charge, size characteristics, hydrophobicity and biological activity. In this work, it was observed that hydrolysates of melon seed (*Cucumis melo L.*) storage proteins exhibit antifungal and antioxidant biological activities.

Methodology. Melon storage proteins were extracted based on their solubility. Protein fractions were proteolyzed with different enzymes (trypsin, proteasa K, pepsine, alcalasa). The peptide screening was achieved by molecular exclusion chromatography (EC) and ionic exchange chromatography (IEC). Antifungal activity essays were developed by ~~-~~poisoned agar" method against *A. alternata, F. solani, Trichoderma y A. Niger* for vegetative growth inhibition. Same method was applied to test sporulation inhibition capacity using 5x103 conidia/mL de *Trichoderma y A. Niger.* Peptide fractions were tested individually and in combination. Free radical scavenging activity employing the method of decoloration of DPPH was determined for antioxidant activity.

Results y Discussion. Hydrolysates of albumin and globulin fractions showed antifungal and antioxidant activity. The conditions of effective sequential hydrolysis were the same for both fractions: partial hydrolysis with trypsin [1: 100] at 37 ° C, 1 hr; partial hydrolysis with protease K [1: 100] at 37 ° C, 30 min.

Tab.1. Vegetative fungal growth inhibition of *F. Solani.* Treatment with EC albumin and globulin fractions

Protein Fraction (EC)	Mycelium growth (mm)
Control (-)	30 ± 0.0
A_1	28 ± 1.0
A_2	27 ± 0.5
A_1+A_2	24 ± 2.0
G	15 ± 0.8
G+A_1	10 ± 1.0
G+A_2	9 ± 0.5

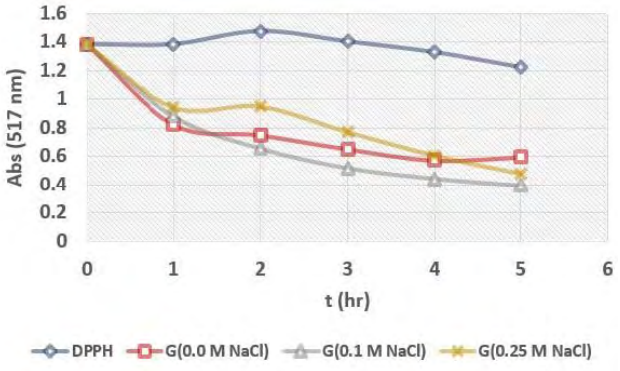

Fig.1. Free radical scavenging activity cynetics. Method of decoloration of DPPH. Treatment with IEC globulin fractions

Conclusions. Peptides obtained from globulin fraction exhibit ≥50% of fungal growth inhibition. Globulin hydrolysates separated by IEC, show 50-70% of DPPH scavening. Albumin and globulin hydrolysates obtained from melon seed, are potential sources of antifungal and antioxidant peptides wich could be used for the development of alternative antimicrobial and antioxidant agents for crop care and disease prevention.

Transcriptome Sequencing by RNAseq and Differential Expression analysis to identify genes involved in Fatty Acids and Alkamides in the Mexican multifunctional plant *Heliopsis longipes*

Genesis V. Buitimea-Cantua[1,2], and Jorge Molina-Torres[1*]

[1] Centro de Investigacion y de Estudios Avanzados. Km 9.6 Libramiento Norte Carretera Irapuato-Leon C.P 36821 Irapuato, Guanajuato, Mexico. Tel. (52)-462 6239600
[2] Tecnologico de Monterrey, Centro de Biotecnologia-FEMSA, Escuela de Ingenieria y Ciencias, Campus Monterrey. Av. Eugenio Garza Sada 2501, Monterrey, N.L., C.P. 64849, Mexico.
*jmolinat@ira.cinvestav.mx

ABSTRACT

Heliopsis longipes is a Mexican endemic multifunctional plant with interesting pharmacological properties, characterized by the synthesized and accumulation of alkamides to high levels in roots. Affinin is the major bioactive compound. Alkamides are low molecular weight (<400 uam) *N*-substituted amides with an α-unsaturated acyl chain.
A previously proposed biosynthetic pathway suggests that alkamides acyl chain derives from oleic acid (C18:1) by successive b-oxidations, dehydration, and isomerizations. To better understand the alkamides biosynthesis pathway, we obtained transcriptome by RNAseq from the root and leaf tissues of *H. longipes*. *De novo* assembly was performed and putative functions were based on BLAST searches against annotation databases including GO and KEGG. The differential gene expression analysis between *H. longipes* tissues detected, 1,399 down-regulated transcripts in *H. longipes* roots and 659 transcripts up-regulated. 71 genes related to the fatty acid synthase (FAS) were identified highly expressed in *H. longipes* leaves, the tissue alkamides non-producer. After comparing the transcriptomes, we found a set of genes whose expression was enriched in the roots, the tissue that accumulates the metabolites in study and they were considered as putative genes encoding for alkamides biosynthetic enzymes. These genes were selected using two criteria: a) A high level of expression in the tissue and species where alkamides are produced, compared to non-producing tissues; and b) The functional annotation. The differential expression profiles show a significant correlation with the accumulated levels of alkamides in the different tissues, suggesting their role in the coordination of metabolite biosynthesis and accumulation, however, they were not correlated with the fatty acid genes. The transcriptomic dataset and analyses presented here lay the foundation for further research on this important endemic plant species.

Phenolic compounds accumulation during postharvest life in the peel of six mango cultivars.

Juan Luis Monribot-Villanueva[1a], José Miguel Elizalde-Contreras[1a], Jiovanny Arellano de los Santos[1], José Antonio Guerrero-Analco[1]*, Martín Aluja[2], Eliel Ruiz-May[1]*

[1]Red de Estudios Moleculares Avanzados, [2]Red de manejo Biorracional de Plagas y Vectores. Instituto de Ecología A. C., Clúster BioMimic®, Carretera Antigua a Coatepec 351, El Haya, CP 91070 Xalapa, Veracruz, México.

[a]Authors contributed equally to this work. *Corresponding authors: eliel.ruiz@inecol.mx, joseantonio.guerrero@inecol.mx. Tel: (228) 842 1800.

A great interest has been paid to use mango fruits byproducts such as peel and kernel due to its high content of fiber and antioxidant compounds. Mango fruits are collected at the mature green stage, stored and ripened during commercialization. During this, several structural and molecular changes happened in the fruit. However, it has been poorly studied the content of antioxidant compounds in mango peels during shelf life (SL). In this study, we screened the content of phenolic compounds in mango skins during the first six days of shelf life in six mango cultivars (Criollo, Atkins, Manililla, Manila, Ataulfo and Kent). We approached our study by using ultra high-performance liquid chromatography coupled with tandem mass spectrometry (LC-ESI-MS-MS) and dynamic multiple reaction monitoring (dMRM) as acquisition method of the spectrometric fingerprints. We searched 60 different phenolic compounds and we found Gallic acid, Mangiferin, (+)-Catechin, (-)-Epicatechin, Quercetin, Kaempferol-3-O-glucoside, Quercetin-3-D-galactoside and Quercetin-3-glucoside. Most of them with a negative trend along SL. Also we performed an untargeted metabolomics analysis in order to identify chemical biomarkers associated to the ripening process along SL and we found increased levels of one sphingolipid that could be participating as signal molecule in the ripening process. The analysis of the results allowed us to determine mango peels as a good source of antioxidant molecules or nutraceutical products.

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Molecular cloning, heterologous expression and structural modeling of Glucose-6-phosphate dehydrogenase from the protozoan *Giardia lamblia*

Laura Eloísa Morales-Luna[1], Saúl Gómez-Manzo1, Jaime Marcial-Quino[2], Ortíz-Ramirez Paulina[1], Segundo-Liberato Marisol[1], Yadira Yazmin Cortes Morales[1], Erick Alcaraz-Carmona[1], Fabiola Fernández-Rosario[1], Víctor Martínez-Rosas[3], Laura Eloísa Morales-Luna[1], Edson Jiovany Ramírez-Nava[1], María Fernanda Torres-Rojas[1], 4Abigail González Valdez, Paulina Hernández Ochoa[1], Beatriz Hernández Ochoa[5].
[1]Instituto Nacional de Pediatría. Laboratorio de Bioquímica Genética. SS, CD.MX, Mexico.
[2]CONACYT-Instituto Nacional de Pediatría, SS, CD.MX. Mexico.
[3]Escuela Nacional de Ciencias Biológicas, IPN, CD.MX, Mexico.
[4]Departamento de Biología Molecular y Biotecnología, Instituto de Investigaciones Biomédicas, UNAM,CD.MX, Mexico.
[5]Laboratorio de Inmunoquímica, Hospital Infantil de México Federico Gómez, CD.MX, Mexico. E-Mail: elomorlu@gmail.com;saulmanzo@ciencias.unam.mx

Glucose-6-phosphate dehydrogenase (G6PD) is the first enzyme in the pentose phosphate pathway and is highly relevant in the metabolism of *Giardia lamblia.* Previous reports suggested that the G6PD gene is fused with the 6-phosphogluconolactonase (6PGL) gene (*6pgl*). Therefore, in this work, we decided to characterize the fused G6PD-6PGL protein in *Giardia lamblia.* First, the gene of *g6pd* fused with the *6pgl* gene (*6gpd*::*6pgl*) was isolated from trophozoites of *Giardia lamblia* and the corresponding G6PD::6PGL protein was overexpressed and purified in *Escherichia coli*. The protein has three conserved motifs: **RIDHYLGKE**, **GxxGDLA**, and **EKPxG** that are associated with the correct positioning of the substrate (G6P) and coenzyme (NADP+) during enzymatic reaction. Then, we characterized the native oligomeric state of the G6PD::6PGL protein in solution and we found a catalytic dimer with an optimum pH of 8.75.

Furthermore, we determined the steady-state kinetic parameters for the G6PD domain and measured the thermal stability of the protein in both the presence and absence of guanidine hydrochloride (Gdn-HCl) and observed that the G6PD::6PGL protein showed alterations in the stability, secondary structure, and tertiary structure in the presence of Gdn-HCl. Finally, computer modeling studies revealed unique structural and functional features, which clearly established the differences between G6PD::6PGL protein from *G. lamblia* and the human G6PD enzyme,proving that the model can be used for the design of new drugs with antigiardiasic activity. These results broaden the perspective for future studies of the function of the protein and its effect on the metabolism of this parasite, as a potential pharmacological target.

Identification of chemical compounds that bind to human Gpn1 GTPase in computational molecular docking experiments

Julio A. Muñiz Luna, Gema R Cristóbal-Mondragón, Rogelio González-González, Angel Santigago, Nina Pastor, Mónica R. Calera and Roberto Sánchez-Olea.

Instituto de Física, Universidad Autónoma de San Luis Potosí, Av. Manuel Nava #6, Zona Universitaria, C.P. 78290, San Luis Potosí, SLP, México. Tel: (444) 826-2300, ext 5717. Email: rsanchez@ifisica.uaslp.mx

The Gpn1 protein belongs, together with Gpn2 and Gpn3, to the family of GTPases GPN. The three proteins are universally conserved in eukaryotic cells and are essential for life, probably due to their involvement in the nuclear accumulation of RNA polymerase II, the enzyme that transcribes all protein-coding genes. Gpn1 forms a Gpn1/Gpn1 homodimer or a Gpn1/Gpn3 heterodimer. The crystallographic structure of human Gpn1 has not been determined, but the structure of a truncated form of the Gpn1 ortologue in the yeast *Saccharomyces cerevisiae* has been determined, which allowed us to apply modeling and molecular dynamics tools to generate structural models of the human Gpn1 homodimer and the Gpn1/Gpn3 complex. In this work we employed these models and computational techniques of molecular docking to identify small molecules with the ability to interact with Gpn1 at the atomic level. We also tested the effect of the compounds selected in the first stage on the GTPase enzymatic activity of recombinant HisGpn1/Gpn1 and HisGpn1/Gpn3. We also established the experimental conditions to obtain monodisperse human HisGpn1/Gpn1 and HisGpn1/Gpn3 by dynamic light scattering experiments, with the future goal of assessing the effect of the Gpn1-binding drugs on the integrity of the Gpn1 homodimer and Gpn1/Gpn3 heterodimer. The availability of pharmacological inhibitors of Gpn1 will accelerate the identification of the cellular and biochemical processes controlled by this essential GTPase in human cells.

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Common misconceptions in the usage of some evolutionary concepts in protein biochemistry

Ricardo Muñiz Trejo, Sergio Romero Romero, Daniel Alejandro Fernández Velasco

Laboratorio de Fisicoquímica e Ingeniería de Proteínas, Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México Circuito Interior, Av. Universidad 3000, Ciudad Universitaria, Alcaldía Coyoacán, Ciudad de México, C.P. 04510, 5623-2259, ricardo.muniz.trejo@ciencias.unam.mx

Two critical questions in biochemistry guide several research groups all around the globe: How do amino acid sequences determine the three-dimensional structure of a protein and how do these sequences encode a function? To reach an answer, it is essential to address them from an experimental perspective, as well as a historical one, that is, we need to understand proteins 'in the light of evolution.' However, given the controversial nature of evolutionary theory, recent developments in our knowledge of evolution go overlooked by most researchers in protein evolution. This is especially apparent by the pervading adaptationism found in the scientific literature on the topic. To guarantee a better understanding of this process, we need to gain a deeper insight into how some ideas from evolutionary biology fit with our biochemical standpoint. This work delves, from a theoretical perspective, into the crucial question: how some problematic concepts such as constraint and adaptation comprise the structural and functional variation of proteins? To diagnose those problems and limitations, we analyzed different definitions of these concepts and compared them with the use taken in the biochemical literature. We also discuss how to adapt current methodologies used in modern evolutionary biology to assess experimentally the historical processes involved in the evolution of these pivotal macromolecules.

Analysis of the impact of potassium levels on porcine kidney BADH coenzyme binding site

[a] César Muñoz-Bacasehua, [b]Jesus A. Rosas-Rodríguez, [c] Aldo A. Arvizu-Flores, [d]Georgina Garza-Ramos, [a] Elisa M. Valenzuela-Soto*

[a]Centro de Investigación en Alimentación y Desarrollo AC, Carretera a La Victoria Km 0.6, Hermosillo, Son. E.M Valenzuela-Soto: elisa@ciad.mx
[b]UNISON Unidad Regional Sur, Navojoa, Son. [c]UNISON, Unidad Centro, Hermosillo, Son. [d]Depto. de Bioquímica, Fac. de Medicina, UNAM, Cd. de México.

Glycine betaine is the main osmolyte synthesized and accumulated in renal cells; its synthesis is catalyzed by betaine aldehyde dehydrogenase (BADH) using NAD^+ as a coenzyme. Previous studies in porcine kidney BADH (pkBADH) suggested that the enzyme has two kinds of active sites and undergoes potassium-induced conformational changes. The aim of the present study was to determine potassium role in the heterogeneity of pkBADH´s active site. PkBADH was titrated with NAD^+ under variable concentrations of KCl and the interaction measured by Isothermal Titration Calorimetry (ITC), Circular Dichroism (CD) and by fluorescence spectroscopy. PkBADH was modeled using the crystallographic structure of the BADH from *P. aeruginosa* deposited in the PDB (MOE software). Apparent T_m of the CD-thermal transitions was 2 and 4ºC higher at 25 and 100 mM K^+ respectively. ITC data showed different NAD^+ association constants (Kd) to first and second site at 1 mM KCl (51.02 and 8.26 µM respectively), the ΔH_b value to each site was -6.67 and 3.2 kcal mol^{-1}, and ΔS_b values were -2.73 and 34 cal mol^{-1} (respectively). At 75 mM KCl, Kd values to first and second site were 10.02 and 5.78 µM, the ΔH_b value to each site was -28.3 and 3.9 kcal mol^{-1} and ΔS_b values were -71.9 and 37 cal mol^{-1}. Fluorescence spectroscopy data showed tryptophans accessibility was potassium dependent; suggesting that K^+ modify the structure of NAD^+ binding site. Three different potential K^+ binding sites were found in pkBADH. All data suggest slight changes in the conformational ordering of pkBADH active site, which involves a process of compensation of energy between enthalpy and entropy of formation of BADH-K^+-NAD^+ complex.

Function of the C-terminal domain of large-subunit catalases

Teresa Nava Ramírez and Wilhelm Hansberg Torres
Departamento de Biología Celular y del Desarrollo, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México (UNAM), Circuito exterior s/n, Ciudad Universitaria, Colonia UNAM, Delegación Coyoacán, México D.F., C.P. 04510, México. Tel. (55) 56225655, whansberg@ifc.unam.mx

Catalase dismutates hydrogen peroxide and is the main antioxidant response in the presence of high concentrations of H_2O_2. *Neurospora crassa* has three catalases: two large size-subunit catalases (CAT-1, CAT-3), a small subunit catalase (CAT-4), besides a catalase peroxidase (CAT-2). Large-subunit catalases have a carboxylterminal domain of ≥150 amino acids, whose structure is similar to flavodoxine. This domain has been considered as a member of the superfamily of glutamine-amidotransferase type I and the superfamily DJ-1/ThiJ/PfpI. Here we analyze the possible holdase function of the C-terminal domain.

When the carboxyl-terminal of the CAT-1 is eliminated with subtilisin, the protein looses stability when it is incubated at high temperature and also its capability to be activated with detergents. Complete CAT-1, CAT-3 and C50, a dimer of the Cterminal domain of CAT-3, avoided heat denaturation of citrate synthase and alcohol dehydrogenase (ADH) and denaturation of beta-galactosidase with 8 M urea. Protected enzymes retained activity. In contrast, CAT-1 without the Cterminus domain (C63) was unable to protect these proteins from denaturation. TheC63 catalytic domain retained full activity but lost its very high stability at 90°C and its activation by detergents and other denaturing agents. We also implemented a chaperone assay that employs H_2O_2 as a denaturing agent: the Hsp31, C50 and the azide inactivated CAT-3 protected ADH from denaturalization by H2O2 whereas C63 and CAT-A did not.

Hsp31 chaperone has a hydrophobic bowl at its dimer interface that has been proposed to capture partially folded proteins under heat-shock conditions. We evaluated if hydrophobic zones in the C-terminal domain were important for its holdase activity using the bis-ANS probe. Hsp31, CAT-1 and C50 incubated at 45°C with the probe gave an increased signal as compared to room temperature, whereas BSA and the small-subunit catalases, CAT-A of *A. niger* and bovine catalase, gave a low signal under both conditions. This suggests that Hsp31, CAT-1 and C50 exposed hydrophobic zones at 45°C.

These experiments indicate that the C-terminal domain of the large-subunit catalases has a chaperone-holdase activity.

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Escherichia coli regulates the expression of miR-146 and -451 in chorioamniotic membranes in an infection model.

Mercedes Olvera-Valencia[1], Jovana Pérez-Sánchez[1], Janelly E. Palacio-Luna[1], Christian Reyes-Mayoral[2], Graciela Villeda-Gabriel[3], Irma Sosa-González[3], Elsa R. Moreno-Verduzco[4], Mauricio Osorio-Caballero[5], Guadalupe García-López[6], HéctorFlores-Herrera[1].
Departments: Inmunobioquímica[1]., Biología de la Reproducción[2]., Infectología[3]., Subdirección de auxiliares diagnostico[4]., Salud Sexual y Reproductiva[5]., Fisiología y Desarrollo Celular[6].
Instituto Nacional de Perinatología Isidro Espinosa de los Reyes.
Responsible author e-mail: merch.honey@gmail.com (MOV) **Address:** Street Lago Chapala 26, G-605, Anáhuac, Miguel Hidalgo, 11320, Mexico City. **Cel Phone:** 558372-1606

BACKGROUND

Infections during pregnancy induce in the Human Fetal Membranes (HFM) the secretion of different immunological mediators. Recently, the participation of a class of non-coding RNA (miRNAs) involved in the regulation of the inflammatory response has been demonstrated. The main objective was determinate the expression of miR-146 and -451 in HFM during stimulation with *E. coli* in an infection model.

METHODS

Six HFMs were processed and mounted in the Transwell system as previously reported (Osorio-Caballero 2015). Choriodecidua (CHD) or amnion (AM) was stimulated directly with *E. coli* ($10^{2,4,5 \text{ and } 6}$ CFU/mL) during 3, 6 and 24 hours. After this time the culture medium of both the zone stimulation and the opposite zone was recovered and the RNA was extracted by TRIzol . The retrotranscription and the PCR to the final point were carried out with its specific primers. The expression bands were determined by the optical density with the ImageJ program. Data are presented as the mean ± standard deviation and a significant difference ($p \leq 0.05$).

RESULTS

After directly stimulating the CHD tissue with the different concentrations of *E. coli* for 3, 6, and 24 hours we observed an increase in the expression profile of miR-146 and miR-451 with respect to the controls ($p \leq 0.05$). In the same stimulation and time Interval, we observed in the amniotic epithelium increased in the expression of miR-146 and miR-451 with respect to the specific control group ($p \leq 0.05$). When we stimulating in the AM the miR-146 increased in both tissues respect to the controls ($p \leq 0.05$) but miR-451 not responds to stimulations in any faces comparing to the control ($p \leq 0.05$).

DISCUSION

There is a differential expression of miR-146a and -451a between the tissue of the CHD and the AM epithelium which depends on the site of stimulation. In the amniotic epithelium, we did not observe variations in the expression of miR-451a, which is why its functional role as a modulator of the inflammatory response is investigated. These results as a whole include an export mechanism between the miR-146a and the -451a tissue of the CHD and the AM epithelium.

Hypoxia inducible factor -1 regulates key glycolytic genes in WSSV-infected white shrimp

Diana Lourdes Padilla-Bórquez[1], José Arquímides Godoy-Lugo[2], Melissa María Miranda-Cruz[1], Alex Ante-Vázquez[1], Jennifer Joceline Poom-Llamas[1], Jesús Alfredo Rosas-Rodríguez[1], José Guadalupe Soñanez-Organis[1]*.

[1]Universidad de Sonora, Depto. de Ciencias Químico-Biológicas y Agropecuarias, Universidad de Sonora Unidad Regional Sur, Apartado Postal 85880, Navojoa, Sonora, México.
[2]School of Natural Sciences, University of California Merced, 5200 N Lake Road, Merced, CA 95343, USA.
*Corresponding author: Dr. Jose Guadalupe Soñanez-Organis Depto. de Ciencias Químico Biológicas y Agropecuarias Universidad de Sonora Unidad Regional Sur Apartado Postal 85880, Navojoa, Sonora, México Email: jorganis@navojoa.uson.mx or josegpeso@gmail.com

ABSTRACT

Hypoxia inducible factor -1 (HIF-1) is a transcriptional factor that induces genes involved in glucose metabolism and is formed by a regulatory α-subunit (HIF-1α) and a constitutive β-subunit (HIF-1β). In the, HIF-1 regulate key glycolytic genes in white shrimp *Litopenaeus vannamei* exposed to hypoxia. Recently, we demonstrated that HIF-1 participates in the pathogenesis of white spot syndrome virus (WSSV) infection through the regulation of the LDH expression and glucose metabolism in highly energetic tissues of infected shrimp. However, the contributions of HIF-1 to the regulation of key glycolytic genes in WSSV-infected shrimp has been not examined. In this study, the effect of HIF-1 silencing on the expression and activity of key enzymes in glucose metabolism (hexokinase-HK, phosphofructokinase-PFK, and piruvate kinase-PK) tissues of white shrimp infected with the WSSV were studied. The expression for HK, PFK y PK increased in each tissue evaluated during the WSSV infection, translating into increased enzyme activity. Silencing of HIF-1 blocked the increase of HK, PFK and PK expression and enzyme activity produced by WSSV infection. These results demonstrate that HIF-1 up-regulates the expression of key glycolytic genes during WSSV infection and that this induction contributes to substrate metabolism in energetically active tissues of infected shrimp

Sphingolipid biosynthesis and function in bacteria

Jonathan Padilla-Gómez[1], Daniela A. García-Soriano[1], Diana X. Sahonero-Canavesi[1], Sebastian Poggio-Ghilarducci[2], Isabel M. López-Lara[1], and Otto Geiger[1*]

Centro de Ciencias Genómicas, Cuernavaca, Morelos, México1 and Instituto de Investigaciones Biomédicas2, Ciudad de México, México; Universidad Nacional Autónoma de México.
*Av. Universidad s/n, Col. Chamilpa 62210, Apdo. Postal 565-A, Cuernavaca, Morelos, México. Phone Number: (777)3290815, otto@ccg.unam.mx

Sphingolipids are ubiquitous in eukaryotes, where they play essential roles as structural components of cellular membranes and as signaling molecules that participate in a wide range ofphysiological and pathological processes. In contrast, few bacteria are known to produce sphingolipids and their presence is thought to be so exotic that many of them have –*Sphingo*" as prefix in their genus name. However, in some bacteria, sphingolipids might be formed only under certain physiological conditions to resist different types of stress such as acidity or high temperatures. Although the eukaryotic genes and enzymes involved in the sphingolipid biosynthesis are reported, little knowledge exists for bacteria. An exception is the first step, catalyzed by serine palmitoyltransferase (SPT) that condenses serine and a fatty acyl-CoA to form the first intermediate 3-oxo-sphinganine. Phylogenetic analysis suggests that operons for sphingolipid biosynthesis exist in many more bacteria. Upstream of the *spt* gene, some of these operons contain a putative acyl carrier protein (*acp*) gene. This finding suggests that specialized ACPs, instead of coenzyme A (CoA), can be used in some cases during the initial step of sphingolipid biosynthesis in bacteria. Our bioinformatic analysis of the *Escherichia coli* BL21(DE3) genome shows that it contains a gene encoding for a putative SPT (ECD_02854), which is preceeded by a gene encoding a putative ACP (ECD_02853) and they probably form an operon with a putative sugar epimerase (ECD_02852) and a putative acyl-CoA synthetase (ACS, ECD_02851).Downstream from this first operon, there is a second operon which contains genes that are probably required for the sphingolipid transport in *E. coli* BL21(DE3). For example, ECD_02855 and ECD_02856 (LptG and LptF, respectively; putative lipopolysaccharide export system permease proteins) and ECD_02859 (yghQ, a putative inner membrane polysaccharide flippase). From the first operon, we think that three of the genes are required to synthetase the first intermediate in the sphingolipid biosynthesis, 3-oxo-sphinganine. In our simplest model, the putative ACS ECD_02851 would be in fact an acyl-ACP synthetase (Aas), which would convert the ACP ECD_02853 to its acylated form. Then, SPT ECD_02854 would use acyl-ACP ECD_02853 and L-serine to form 3-oxosphinganine, which would then be further modified to produce *E. coli* BL21(DE3) sphingolipids.Studying the biochemical function of specific genes we could show that the predicted ECD_02853 is an ACP, since it carries the 4'-phosphopantetheine prosthetic group. Using the acyl-ACP synthetase (AasS) from *Vibrio harveyi*, it was possible to synthetize the acylated form of the constitutive ACP (AcpP ECD_01090) from *E. coli* BL21(DE3) as observed by urea-PAGE, using fatty acids from C6 to C18. However, acylation of the special ACP ECD_02853 could not be observed so far. Acylation assays of this ACP ECD_02853 will be done with the AasS from *V. harveyi*, using [3H]-labeled palmitic acid. Products will be identified as [3H]palmitoyl-ACP and by urea-PAGE. Also, enzymatic activity of putative SPT ECD_02854 from *E. coli* BL21 (DE3) was studied, using SPT from *Sphingomonas wittichii* RW1 as a positive control. After expression of *spt* candidate gene in *E. coli*, *in vitro* formation of 3-oxo-esfinganine was observed when using palmitoyl-CoA as thioester substrate, in addition to other lipid compounds that are possibly sphingolipids. Enzymatic assays using both palmitoyl-CoA and palmitoyl-ACP ECD_02853 or palmitoyl-AcpP ECD_01090, will be performed to test them as thioester substrates for the different SPTs. Kinetics studies will be used to show if palmitoyl-ACP ECD_02853 is a better substrate for SPT than the CoA or ECD_01090 derivatives.

Structural comparison of human and murine G0S2 proteins

Edgar Daniel Páez-Pérez [a], Ruben Paul Gaytan-Colin [b], Gabriela Montero-Morán [c] and Samuel Lara-González [a] *

[a] IPICYT, Laboratorio de Biología Estructural, Instituto Potosino de Investigación Científica y Tecnológica, San Luis Potosí, México.
[b] Instituto de Biotecnología, Universidad Nacional Autónoma de México, Av. Universidad 1001, Col. Chamilpa, 62210 Cuernavaca, Morelos, México
[c] Facultad de Ciencias Químicas, Laboratorio IBCM, Universidad Autónoma de San Luis Potosí, Manuel Nava 6, Zona Universitaria, San Luis Potosí, SLP, México
**Corresponding author:* Samuel Lara González, Camino a la Presa San José 2055, Col. Lomas 4ta, CP 78216, San Luis Potosí, SLP. Tel: 01(444)8342000 ext 6239, E-mail: samuel.lara@ipicyt.edu.mx

The G0/G1 switch gene 2 (*G0S2*) was originally identified in blood mononuclear cells following induced cell cycle progression. Translation of *G0S2* results in a small basic protein of 103 amino acids in size. Until now, the best-known function of G0S2, is the inhibitory effect that it has on the activity of the lipolytic enzyme, adipose triglyceride lipase (ATGL). Although, G0S2 has also been related to various biological activities, including roles in cell cycle, cell proliferation, apoptosis, inflammation and carcinogenesis. Orthologs of *G0S2* have only been identified in vertebrates including human, mouse, rat and chicken. Mouse G0S2 comprises 103 amino acids and shares 77.7 % sequence identity to the human protein. To date, no experimental three-dimensional structure of G0S2 or any structural study has been published. We performed a structural analysis comparing recombinant human and murine G0S2 proteins. We found by bioinformatic approach that the C terminal region of human G0S2 was predicted to be disorder, in contrast, the same bioinformatic analysis of the mouse G0S2 protein did not show any disorder for the C terminal region of the full sequence. This observation agrees with the data obtained by size exclusion chromatography (SEC) and dynamic light scattering analysis (DLS), our results shows a greater Stock radius and a lower hydrodynamic diameter for the human G0S2 than the murine G0S2.

Progesterone modulates the secretion of heat shock protein (Hsp)-60 in choriodecidua tissue after stimulation with *Escherichia coli* in a model of infection.

Janelly Estefanía Palacios Luna[1]., Mercedes Valencia Olvera[1]., Sandra Lizeth Alvarado Ortiogoza[1]., Christian Reyes Mayoral[2]., Elsa Romelia Moreno Verduzco[3]., Mauricio Osorio Caballero[4], José Carlos Aguilar Carrasco[5], Cecila Helguera Repetto[1]., Héctor Flores-Herrera[1].

Department of: Inmunobioquímica[1], Biología de la Reproducción[2], Subdirección de Asistencia de Diagnostico[3], Salud Sexual y Reproductiva[4]. Fisiología de Desarrollo Celular[5]. Instituto Nacional de Perinatología –Isidro Espinosa de los Reyes". Register number: 2017-2-82 (Granted to HFH).
e-mail: janelunfa@gmail.com (JEPL).

Introduction: Previously, we have reported that *Escherichia coli* induce in the corideciudual tissue (CDH) the secretion of Hsp-60 and -70 [1]. It has been show that progesterone (P4) decreases Hsp-60 through a 10-Kda chaperone-type protein; however, this effects has not been evaluated on CDH tissue.

Objectives: to evaluate the effect of progesterone on the secretion of Hsp-60 in the CDH tissue after stimulation with *Escherichia coli.*

Material and methods: The CDH tissue was processed and mounted in the Transwell system and incubated by 1 hour with P4 (10 mM). After this time, CHD was stimulated with *Escherichia coli* (10^2 y 10^6 UFC/mL) alone or in combination with P4(10 mM), RU486 (10 mM) [2], and P4+RU486 by 3, 6, and 24-hours. After this time, medium was collected and centrifugated and the medium was stored at 70°C. The quantification of Hsp-60 was determined by the ELISA test.

Results: At 3 hours, the concentration of Hsp-60 (1.78 ± 0.13 ng/mL) increased significantly with the addition of *E. coli* 10^2 CFU/mL 1.2- ($p = 0.05$) and 10^6 CFU/mL 1.6-fold ($p < 0.05$). The addition of P4 did not significantly reduce the secretion of Hsp-60. At 6 hours (1.78 ± 0.14 ng / mL) it was significantly increased with the addition of E. coli 10^2 CFU/mL 1.5- ($p \leq 0.05$) and 10^6 CFU/mL 1.6-fold ($p < 0.05$). The addition of P4 did not significantly reduce the secretion of Hsp-60. Finally, at 24 hours (1.97 ± 0.10 ng/mL) *E. coli* significantly increased 10^2 CFU/mL 1.6- ($p \leq 0.05$) and 10^6 CFU/mL 2.5-fold ($p < 0.05$). The addition of P4 significantly reduced the secretion of Hsp-60 with respect to the stimulation with *E. coli* 10^6 CFU/mL ($p = 0.05$).

Conclusions: Although we observed a reduction in the secretion of Hsp-60 it was not statistically significant with respect to the control group; however, the effect of progesterone on the secretion of Hsp-60 was observed at 24-hours.
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Mitochondrial function and dynamics in MH-S alveolar macrophages treated with P27 y PE_PGRS33 recombinant proteins of *Mycobacterium tuberculosis*

Iris Selene Paredes González[1], Omar Emiliano Aparicio Trejo[2], Clara Inés Espitia Pinzón[3], Dulce Mata Espinosa[1], Brenda Marquina Castillo[1], Juan C. León Contreras[1], José Pedraza Chaverri[2], Rogelio E. Hernández Pando[1]

[1] Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. Av, Vasco de Quiroga #15, Col. Belisario Domínguez Sección XVI, Del. Tlalpan C.P.14080, 044 272 701 37 44 iris_selene142@hotmail.com
[2] Facultad de Química, UNAM
[3] Instituto de Investigaciones Biomédicas, UNAM

Background

Mitochondria of Mycobacterium tuberculosis (Mtb)-infected macrophages display several changes regarding ATP production, membrane potential, cytochrome C release, mitochondrial cristae morphology and a tendency to locate around Mtb-containing phagosomes. These changes are associated with mycobacterial strain virulence. Recent evidence shows that specific mycobacterial proteins, such as P27 and PE_PGRS33, target mitochondria in J774 cells, being their precise role in mitochondrial function unknown.

PE_PGRS33 belongs to the PE/PPE (Pro-Glu/Pro-Pro-Glu) protein family and it is expressed during pulmonary TB infection. This protein is able to induce cell death and innate immune response modulation via TLR-2. P27 is a lipoprotein that binds and transports mycobacterial triacylglycerides and has a critical role in lipid metabolism regulation, cell wall integrity, and virulence. Oxidation of transcription factor WhiB4 induces the transcription of PE/PPE genes and impairs antioxidant enzymes production, thus maintaining an oxidizing environment and promoting bacillary replication.

Materials and methods

We used the MH-S (alveolar macrophages) cell line. 10 million cells were incubated with recombinant proteins P27 or PE_PGRS33. The activity of the respiratory chain, mitochondrial membrane potential, and ATP was evaluated by high-resolution respirometry (O2k Oxygraph, OROBOROS, Innsbruck, Austria). In addition, mitochondrial ultrastructure was analyzed by electronic microscopy. Isolated mitochondria were incubated with recombinant proteins (P27 or PE_PGRS33) for antioxidant activity assessment.

Aim

To determine the functional and structural abnormalities of mitochondria induced by mycobacterial proteins P27 and PE_PGRS33.

Exploring chemical space to find potential inhibitors of protein tyrosine phosphatase 1B.

Luis Enrique Pérez del Castillo, Claudia Isela Avitia Domínguez, Marcelo Gómez-Palacio Gastelum, Jorge Cisneros Martínez, Alfredo Téllez Valencia
Facultad de Medicina y Nutrición Universidad Juárez del Estado de Durango Av. Universidad s/n, centro C. P. 34000, Victoria de Durango, Durango, México. Tel: 6188121687, atellez@ujed.mx

Abstract

Diabetes is a group of metabolic diseases characterized by hyperglycemia as a result of defects in insulin secretion, insulin action or both. Type 2 diabetes is the most prevalent worldwide. Protein tyrosine phosphatase 1B (PTP1B) acts as a negative regulator in the insulin signaling cascade, and is considered as a good target for the design of new drugs against this disease. However, in the PTPs family, the protein tyrosine phosphatase of T cells (TCPTP) has a 74% homology in its catalytic site with PTP1B, situation that creates a challenge for drug design. Recently, specific sites (A, B, C, D) within the catalytic site have been reported in PTP1B, interactions in these sites can give selectivity with respect to TCPTP. Considering the above, a chemical dataset of 24,002 small molecules from Chembridge library was studied by virtual screening using AutodockVina and Glide software. First, the dataset was filtered according to the rules by Veber, Lipinski and Egan, obtaining 7,706 compounds.The molecules were docked into PTP1B in both open and closed conformations. Potential inhibitors were selected according to their binding energy and interactions in sites A, B, C, and D in PTP1B and their binding mode in TCPTP. Results in AutodockVina showed that the best compounds were **7944843**, **39580720**, and **54765178** that made hydrogen bonds with residues Arg24 (site B), Tyr46 (site C), and Lys120 (site D). In the case of Glide, the compounds were **73116554**, **40101864**, and **7862790** that formed hydrogen bonds with Arg24 (site B), Asp48 (site C), Asp181 (site D), Tyr46 (site C) and Ser216 (site D). In conclusion, these molecules have the potential to inhibit PTP1B in a selective manner.

Biochemical Characterization of Two Single Mutant (A+ and Nefza) that Give Rise to a polymorphic Glucose-6-phosphate dehydrogenase A- Double Mutant.

Edson Jiovany Ramírez Nava[1], Víctor Martínez Rosas[1], Yadira Yazmín Cortés Morales[1], Laura Eloísa Morales Luna[1], Erick Alcaraz Carmona[1], Fabiola Fernández Rosario[1], María Fernanda Torres Rojas[1], [3]Abigail González Valdez, [4]Beatriz Hernández Ochoa, Paulina Hernández Ochoa[1], Jaime Marcial-Quino[4] and Saúl Gómez-Manzo[1].

[1]Instituto Nacional de Pediatría. Laboratorio de Bioquímica Genética. SS, CD.MX. [2]CONACYT-Instituto Nacional de Pediatría, SS, CD.MX. [3]Departamento de Biología Molecular y Biotecnología, Instituto de Investigaciones Biomédicas, UNAM, CD.MX. [4]Laboratorio de Inmunoquímica, Hospital Infantil de México Federico Gómez, CD.MX. Mexico. E-Mail: edsonjiovany@ciencias.unam.mx; saulmanzo@ciencias.unam.mx

Abstract:
Glucose-6-phosphate dehydrogenase (G6PD) is a key regulatory enzyme that plays a crucial role in the regulation of cellular energy and redox balance. Mutations in the gene encoding G6PD cause the most common enzymopathy that drives hereditary nonspherocytic hemolytic anemia. To gain insights into the effects of mutations in G6PD enzyme efficiency, we have investigated the biochemical, kinetic, and structural changes of three clinical G6PD variants, the single mutations G6PD A+ (Asn126AspD) and G6PD Nefza (Leu323Pro), and the double mutant G6PD A− (Asn126Asp + Leu323Pro).
The mutants showed lower residual activity (≤50% of WT G6PD) and displayed important kinetic changes. Although all Class III mutants were located in different regions of the three-dimensional structure of the enzyme and were not close to the active site, these mutants had a deleterious effect over catalytic activity and structural stability.
The results indicated that the G6PD Nefza mutation was mainly responsible for the functional and structural alterations observed in the double mutant G6PD A−. Our study showed that biochemical and structural changes found in G6PD Nefza and A- variants matched those reported for Class I G6PD variants, suggesting the need to re-classify these mutants which should include clinical and biochemical characteristics of these G6PD variants. From the solved three-dimensional structure of the human G6PD protein, we defined changes in the interactions of the amino acid that offer a molecular explanation for the effects of these mutations, and provide a molecular explanation for clinical manifestations observed in individuals with G6PD mutations.

Functional Studies of *SRD5A2* Gene Mutations, Identified in Individuals with Steroid 5α-Reductase Type-2 Deficiency/46,XY Disorder of Sexual Development.

Luis Ramos Tavera, Lizette Mares Gómez, Bertha Alicia Chávez Cano and Felipe de Jesús Vilchis Uribe. *Department of Reproductive Biology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, México City, 14080, México. Tel. 54870900 ext. 2423 vilchisuf@prodigy.net.mx*

Steroid 5α-reductase type-2 isozyme (SRD5A2) plays a determinative role in masculinization of external genitalia during sexual differentiation. Mutations in the *SRD5A2* gene are the cause of steroid 5α-reductase type-2 deficiency and represent the second most common gene alteration of disorders of sexual development 46,XY (46,XY DSD). Currently there are reported more than 60 different pathogenic variants of *SRD5A2,* however little information is available about their impact on the catalytic properties of this isozyme. Here, we determined the effect of point mutations on the kinetic properties (K_m and V_{max}) of *SRD5A2* from patients 46,XY with the enzyme deficiency. PCR-SSCP assays revealed 10 distinct gene variants and sequencing analysis confirmed *SRD5A2* mutant sequences, namely (p.V3I, p.S14R, p.A52T, p.F118L, p.R145W, p.R171S, p.L226P, p.F229S, p.S245Y, p.A248V). Ten mutant constructs and three double-gene variants (p.A49/V89, p.T49/L89, p.T49/V89) were re-created by site directed mutagenesis and expressed in HEK293 cells. Functional studies of the mutant cDNAs demonstrated that 12 of the 13 variants led to partial (V_{max} 56-660 pmol/mgP/h) or complete loss (p.S14R, p.R171S and p.L226P) of 5α-reductase activity, whereas one double-gene variant (p.T49/L89) showed gain-of-function (V*max* 1299 pmol/mgP/h) compared with wild-type enzyme (V_{max} 992 pmol/mgP/h). The missense variants in *SRD5A2* gene were predicted to be pathogenicity-associated mutations by multiple bioinformatic approaches. Together, these data suggest that missense variants associated with 5α-reductase type-2 deficiency primarily affect the catalytic efficiency ($V_{max}/_{Km}$) or result in low residual enzymatic activity. Although the three dimensional structure of human 5α-reductase type 2 is still inconclusive, the alterations reported in the enzymatic activity of 5α-reductase due to mutations in the *SRD5A2* gene, could be explained through erroneous electrostatic interactions between the amino acid residues of enzyme and the substrate testosterone or the NADPH cofactor.

Role of the aryl hydrocarbon receptor (AhR) on the oncoprotein E7-HPV16 degradation mechanism by the proteasoma 26S pathway.

Claudia Vanessa Arellano Gutiérrez[1], Samantha Dayanne Galván Reyes[2], Lorena Ignacia Rodríguez Páez [1],Sofia Lizeth Alcaraz Estrada [3], Gabriela Figueroa González [4], Octavio Daniel Reyes Hernández.[5]*

1.- Departamento de Bioquímica, Laboratorio de Bioquímica Farmacologica, Escuela Nacional de Ciencias Biológicas, del Instituto Politécnico Nacional, Campus Lázaro Cárdenas. 2.-Escuela Superior de Medicina del Instituto Politécnico Nacional, Campus Lázaro Cárdenas, 3.- División de Medicina Genómica. Centro Nacional 20 de noviembre, ISSSTE. 4.- Subdirección de Investigación Básica,Instituto Nacional de Cancerología. 5.- Facultad de Estudios Superiores Zaragoza, Universidad Nacional Autónoma de México,Batalla 5 de mayo s/n esq. Fuerte de Loreto Col. Ejército de OrienteDel. Iztapalapa, C.P. 09230Ciudad de México, México Tels.56230700 y 56230500. Ext. 83201.

maiden_sp@yahoo.com.mx

In Mexico, cervical cancer (CC) represents the second cause of female mortality with an incidence of 13,960 and mortality of 4,769 cases per 100,000 inhabitants. The main factor associated with the development of CC and its precursor lessions is the Human Papilloma Virus (HPV). There are more than 100 HPV genotypes divided in two groups, near 50% cases have HPV16 infection and only 20% have HPV18. All cancerous tissues, transformed by HPV, express oncoproteins E6 and E7, derived from the genome of the HPV; the latter has a high transforming capacity, blocking cellular proteins such as tumor suppressor (pRB). The E7 and pRB interaction disrupts the transcription regulatory complex: pRB/E2F. Upon dissociation of the pRB/E2F complex, the activated E2F promotes the synthesis of cellular products and the progression of the cell cycle, from G1 to S phase. There is evidence that E7 is downregulated through the Ubiquitin Proteasome System (SUP) pathway. In the present work the expression of AhR, Ube2l3, pRB, and p53 were measured in the C33A, HeLa, and SiHa cell lines, observing a significant expression levels trend among cell lines with or without AhR activation.

Conclusions

Semi-quantitative RT-PCR was carried out, and the Ube2l3 expression were constant in the three cell lines, while pRb, p53 and CYP1A1 was greater in the C33A line than SiHa and HeLa lines By the other hand, the AhR gene expression was higher in the SiHa cell line, there was a correspondence with the immunodetection of the AHR protein levels. Previous results suggest that genes involved in the pathway (AhR, pRb and Ube2l3) are present in the three cell lines, however, it is necessary to activate them through several ligands in order to determine their participation in the E7 degradation pathway.

Plasma homocysteine levels in BALB / c mice with L5178Y lymphoma

Fabiola Rodríguez Arévalo [1], Ana María Puebla Pérez[1,2], Jorge Iván Delgado Saucedo [1,2], Martha Patricia Gallegos Arreola[3].

[1]Departamento de Química y [2]Departamento de Farmacobiología, Centro Universitario de Ciencias Exactas e Ingenierías, Universidad de Guadalajara. Blvd. Marcelino García Barragán N° 1421, esq. Calzada Olímpica, C.P. 44430, Guadalajara, Jal. 3División de Genética, Centro de Investigación Biomédica de Occidente, Instituto Mexicano de Seguro Social, Guadalajara, Jalisco 44340, México.
qfb.fabiolara@gmail.com

Elevated levels of homocysteine (Hcy) have been associated with cardiovascular and neurodegenerative diseases. Hcy arises from the action of methionine adenosyltransferase on methionine (Met), followed by conversion to S-adenosylmethionine which acts as the main donor of methyl groups. Once the Hcy is formed, it can follow two routes: remethylation, to transform again to Met, with the help of vitamins of group B; or follow the route of transulfurization to produce cysteine (Cys) through the action of the enzymes cystathionine-β-synthase and cystathionine γ-lyase. There is evidence that Hcy levels are associated with tumor development, however, there are controversies about it. In this work we report the results of plasma Hcy levels on mice with L5178Y lymphoma at different stages of tumor development. To this aim, five groups of mice were formed (n = 5): healthy control group, groups with 5, 10, 15 and 20 days of tumor evolution (inoculated with $2x10^4$ L5178Y lymphoma cells), respectively. The samples were analyzed by high performance liquid chromatography (HPLC), using a C18 column, mobile phase: acetates buffer, flow 1.8 mL/min and deribatization with SBDF at an excitation wavelength of 385 and 515 nm emission. The data were analyzed by ANOVA (SPSS ver.10) and the following results were obtained: 11.30 ± 1.58 µmol/L for the healthy control group, 25.36 ± 3.48 µmol/L, 22.49 ± 7.38 µmol/L, 9.64 ± 2.79 µmol/L and 46.35 ± 27.25 µmol/L for the groups of 5, 10, 15 and 20 days of tumor evolution, respectively. We will continue to evaluate the correlation between Hcy and other metabolites related to its biosynthesis, such as Met and Cys in this experimental model

"Biochemical caracterization for the enzyme *Kl*Bat1, a branched-chain amino acid transaminase in *Kluyveromyces lactis*."

Escudero-Reyes[1], J. Raul; Aguirre-Lopez, Beatriz[1]; Gonzalez, Alicia[1]
[1)] Departamento de Bioquímica y Biología Estructural, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, México City, México; 04360; 5556225631 (Ext. 25631)
amanjarr@ifc.unam.mx

The yeast *Kluyveromyces lactis* is a species that arose before the hybridization and whole genome duplication event which gave rise to the *Saccharomycetae*. Accordingly, *K. lactis* is considered as an ancestral-type organism, similar to the one which formed part of the hybrid from which *S. cerevisiae* derived. Some of the duplicated genes in *S. cerevisiae* that were originated from allopolyploidization are involved in nitrogen metabolism and have their respective orthologous genes in *K. lactis*. *ScBAT1* and *ScBAT2* constitute a pair *S. cerevisiae* paralogous genes generated by allopolyploidization whose products participate in branched-chain amino acid metabolism, *KlBAT1* is the single *K. lactis ScBAT1* and *ScBAT2* orthologous gene. In *S. cerevisiae*, each member of the *KlBAT1/2* gene pair codifies for a branched-chain amino acid aminotransferase (BCAT) carrying out the last step of the biosynthesis and the first one in the catabolism of branch-chain amino acids (BCAAs): valine, isoleucine and leucine (VIL), their respective ketoacids are α-ketoisocaproate, α-keto-β-metilvalerate and α-ketoisovalerate. The VIL amino acids are hydrophobic and have a pivotal role in the formation of protein tertiary structure.

We have previously reported that the orthologous *KlBAT1* encoded protein is a bifunctional enzyme participating in the biosynthesis and catabolism of BCAA. The present work will address the purification and kinetic characterization of *K. lactis* ancestral type isozyme which has 89% identity with *Sc*Bat1 and 79% with *Sc*Bat2. We will discuss *Kl*Bat1 kinetic properties and oligomeric organization as compared to those of *Sc*Bat1 and *Sc*Bat2.

Changes in the transcription of autophagy genes and proteases in aged cells of yeast

René Abraham Mejía Cahuantzi[1], Juan C. Benítez Serrano[2], Guadalupe Rojas Sánchez[1], Carolina Morán Raya[1], Lourdes Millán Pérez Peña[1], Jorge Ramírez Salcedo[3], Irma Pilar Herrera Camacho[1], Nora Hilda Rosas Murrieta[1].
[1]Instituto de Ciencias, Benemérita Universidad Autonóma de Puebla. Edif. IC7, Ciudad Universitaria, San Manuel, Puebla. CP. 72570. Puebla. 22295500 ext. 7283.
nora.rosas@correo.buap.mx
[2]Fac. Ciencias Químicas, BUAP. Edif. FCQ9. Ciudad Universitaria, San Manuel, Puebla. CP. 72570.
[3]Unidad de Microarreglos de DNA, IFC, UNAM. Ciudad Universitaria. México D.F. C.P. 04360

Autophagy is an indispensable cellular process to maintain cellular homeostasis, it is involved in protein turnover, in the selective elimination of macromolecular complexes, organelles and in cell protection to stress. In the present work, the mRNA level of autophagy and protease genes in aged cells of the yeast *Schizosaccharomyces pombe* (*S. pombe*) was analyzed. The morphological changes were evaluated by fluorescence microscopy between days 2 to 11 of cell culture. On day 8 of chronological aging (CLS) of the yeast, the total RNA was extracted and the heterologous hybridization was performed on a DNA microarray of the yeast *Saccharomyces cerevisiae*. Some autophagy genes were verified by qRT-PCR. The analysis of the data made in the GenArise program showed that 65 genes of autophagy or related to the process do not modify their expression: ATG33, ATG9, ATG4, ATG5, KOG1, VTC2, ATG31, ATG23, ATG27, ATG17, ATG11, ATG10, ATG20, ATG18, ATG19, ATG3, PIK1, ATG15, ATG32, VAC8, ATG2, ATG16, ATG7, CUE5, ATG14, ATG21, TG8, ATG13, SNX4, SMF1, SNF3, ENB1, VTC4, MDL1, RGT2, MRS4, MRS3, VPS73, MDL2, SMF2, TAT1, QDR3, PXA2, TPO5, FAT1, KCH1, CTR2, ARN2, PET8, PHO91, AGC1, VTC3, SNQ2, SPF1, ADY2, MAL11, GGC1, ALP1, SUL1, MFM1, HNM1, FET4, OPT1, RSB1. The analysis of qPCR showed that the ATG4, ATG1, ATG4, ATG8 and ATG18 genes do not change their expression in aged cells. The genes that increased were ATG16, ATG38 and ATG22. The genes that decreased were ATG12, TAP42. The RNA level of 44 genes encoding proteases is not modified. In cells aged on day 8 of CLS, autophagy suffers little modification.

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Modification in the mRNA level of transporter coding genes in aged cells of yeast

Nora Hilda Rosas Murrieta[1], René Abraham Mejía Cahuantzi[1], Marisela Coyotl Huexotl[1], Lourdes Millán Pérez Peña[1], Jorge Ramírez Salcedo[2], Irma Pilar Herrera Camacho[1].

[1]Centro de Química, ICUAP, Benemérita Universidad Autonóma de Puebla. Edif. IC7, Ciudad Universitaria, San Manuel, Puebla. CP. 72570. Puebla. 22295500 ext. 7283.
nora.rosas@correo.buap.mx
[2]Unidad de Microarreglos de DNA, IFC, UNAM. Ciudad Universitaria. México D.F. C.P. 04360

Most of the world undergoes a demographic transition process that shows a clear tendency towards the aging of the population. Cell aging and its analysis at the molecular level is a subject of great interest. The objective of the work was to know the set of genes related to intracellular transport whose expression is modified in aged cells of the yeast *Schizosaccharomyces pombe* (*S. pombe*). *S. pombe* was maintained until day 8 of chronological aging. The total RNA was extracted from these cells and the heterologous hybridization was performed in a DNA microarray of *Saccharomyces cerevisiae*. The analysis of the data made in the GenArise program showed 129 genes whose mRNA increases, while 196 decrease and more than 1000 whose RNA do not change. In relation to the transporters, 14 genes increased (MCH1, ITR1, HXT16, AVT3, CCC1, NUP159, POM34, TOM20, THI7, TRK1, VCX1, NUP85, ATG22, NMD3), 18 genes decreased (TOK1, GIT1, NUP49, FTR1, TIM12, NUP1, ERC1, YRB1, FTH1, YRB2, PHO88, AVT7, PRE8, MSN5, ADP1, MDM38, VMA16, ECM1) and 44 genes showed no major changes (VHC1, PDR15, HXT1, AVT6, RPT4, PDR12, PDR5, PEX17, ALR1, GNP1, VBA4, SXM1, MMT2, AVT5, AVT2, VBA4, PHO89, FCY2, SAL1, ZRG17, NUP145, MID1, TPN1, AVT1, SFC1, HXT10, MEP2, HXT3, YHC3, STV1, PHO87, FCY21, PIC2, ZRC1, AGP1, YHK8, AAC3, CTR1, ENA2, FCY22, HOL1,VMR1, THI74). Finally, the mRNA level of the VAC7 vacuolar gene was analyzed by qRT-PCR, confirming that there is no change in the level of that transcript as reported in the microarray. The analysis performed interestingly showed that the number of transporter genes that increase or decrease their expression is lower compared to those in which there are no changes, it is likely that in aged cells only those that require strict regulation are modified, which already can not be sustained or that do not compromise cellular viability, however this will have to be checked in subsequent work.

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Betaine Aldehyde Dehydrogenase is regulated during WSSV infection in white shrimp

Norma Aurora Stephens Camacho[1], José Guadalupe Soñanez-Organis[2], Melissa María Miranda-Cruz[2], Jesús Alfredo Rosas Rodríguez[2]*

[1]Universidad Estatal de Sonora (UES), Lic. Nutrición Humana, Periférico Sur y Carretera Internacional a Huatabampo km 5, S/N, Colonia Juárez, Navojoa, Sonora, México [2]Depto. de Ciencias Químico-Biológicas y Agropecuarias, Universidad de Sonora Unidad Regional Sur, Navojoa, Sonora, México.

*Corresponding author. Email. jrosas@navojoa.uson.mx

The white spot syndrome virus (WSSV) is one of the most lethal viruses that infects shrimps, generating severe economic losses in the aquaculture sector. Despite the advance in the area, to date, there is no effective treatment for the white spot disease (WSD). The osmotic stress is one of the strategies applied to suppress the virus replication and therefore reduction of the mortality of shrimp during infection. The increase in salinity contributes to a better immune response in WSSV-infected shrimp. However, the mechanism that contributes to survival is still unclear, and the information generated by distinct investigations could be contradictory. Betaine aldehyde dehydrogenase (BADH E.C. 1.2.1.8) is present in shrimp and its regulated by salinity variation, suggesting the enzyme participation in the osmotic stress adaptation of white shrimp by the accumulation of the osmolyte glycine betaine. However, differentially gene expression studies in shrimp during WSSV infection focused on the change of the energy production and cellular defense genes, not considering the osmotic response genes. In this work, we evaluate the mRNA levels and activity of BADH enzyme during WSSV infection in white shrimp. Our results indicate that BADH activity was increased 4.28 and 4.59-fold at 24 and 48h post-infection, respectively. The results suggest that BADH could participate in the response for the WSSV infection in white shrimp.

"Characterization of the human solute carrier SLC16A11 and its sequence variant present in Type 2 Diabetes"

Hilda Sánchez Vidal, Gerardo Gamba Ayala, Viridiana Olin Sandoval.
Department of Food Science and Technology, Instituto Nacional de Ciencias Médicas y Nutrición, Vasco de Quiroga 15, Belisario Domínguez, 14080 Mexico City CDMX;
viridiana.olins@incmnsz.mx; Tel. 54 87 09 00 ext. 1575.

Type 2 Diabetes (T2D) is a chronic disease of great importance around the world. In Mexico, it is the first cause of death in adult women and the second among men (1). T2D is caused by a complex interaction of genetic, epigenetic and environmental factors (5). Over the last years there has been described around 40 single nucleotide polymorphisms (SNPs) showing genomic associations with T2D (3). Recently, the presence of five SNPs in the human solute carrier *SLC16A11* gene was identified as a risk haplotype for T2D in Mexican and Latin American populations (6).

SLC16A11 belongs to the *SLC16* gene family that encodes the proteins known as "monocarboxylate transporters" (2). Previous studies showed that this transporter is located in the plasma membrane when associated with the chaperone protein basigin (4). Besides, despite it has been shown that SLC16A11 can transport pyruvate on a proton-coupled reaction, its primary substrate is still unknown (4). Metabolomic analysis have shown that metabolites from lipid metabolism accumulate when the expression of SLC16A11 is modified in cells (4;6). Thus, our hypothesis is that the primary substrate of this transporter could be a metabolite related to lipid metabolism.

The identification and characterization of SLC16A11 with its primary substrate will lead us to understand its function in cells, and how the presence of the 5 SNPs in the T2D-risk variant can contribute to the development of T2D. Therefore, the aim of this project is to express and characterize the SLC16A11 transporter and its T2D-risk variant in *Xenopus laevis* oocytes. So far, we have cloned the *SLC16A11* gene in an expression vector for oocytes and added the Flag tag by site directed mutagenesis. The expression of the SLC16A11 in the oocytes plasma membrane was verified by Western blot. We are now working on the identification of the primary substrate, so that we can kinetically characterize this transporter. Besides, we are currently inserting the sequence modifications to the reference *SLC16A11*, in order to obtain the T2D-risk variant that will be expressed in *Xenopus* oocytes and characterized.

Moreover, with the aim of understanding the structure of SLC16A11, we built a homology-model of SLC16A11 using the software SWISS-MODEL, where we corroborated that this transporter has a similar topology to monocarboxylate transporters.

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STRUCTURAL CHARACTERIZATION OF THE HEAVY CHAIN OF DYNEIN

Santiago Sánchez Saúl[1], Morales Ríos Edgar[1]
[1]Lab. 2, CINVESTAV. Av. IPN No. 2508, San Pedro Zacatenco 07360, CDMX, México. Tel.: 5747-3800 Ext. 5229. E-mail of contact:
edgar.morales@cinvestav.mx, saul.santiago@cinvestav.mx

Human cytoplasmic dynein-1 (hereafter dynein) is a molecular motor of a molecular weight of 1.4 MDa in its homodimeric form. This complex is composed of 6 subunits[1]. Dynein, together with its cofactor, dynactin, transports cargos in a retrograde direction (traffic from nucleus to the cytoplasmic membrane). It participates in the transport of vesicles, organelles, some viruses and during mitosis[2, 6]. Recently, the mid-resolution structure of the complete dynein in its phiparticle auto-inhibited conformation has been solved by cryo-electron microscopy (Cryo-EM) at 8.4 Å resolution[4]. In this structure, it has been possible to solve the secondary structure of the N-Terminus of the heavy chain's tail[4]. This region is essential for dynein interaction with dynactin. Dynactin recruits one dynein when it binds to BICD2, this complex was solved at 8.2 Å of resolution[3]. However, the cargo adaptors HOOK3 and BICDR1 are able to bind two dyneins to dynactin, this complexes were solved to 6.7 and 3.5 Å of resolution respectively. This arrangement results in a dynein–dynactin complex that moves faster and can produce larger forces when compared with complexes containing a single dynein.

Thus, cargo can control the output of the dynein-dynactin machine via the identity of its activating adaptor[5]. Since there is no high-resolution structure that allows us to visualize in more detail the binding of dynein with the cargo adaptor and the cofactor dynactin, the aim of our project is to obtain the atomic structure of a fragment of the N-terminal tail domain of the heavy chain from dynein by X-ray crystallography.

We started with a fragment of residues 1-572 of the heavy chain, which was truncated from the terminal carboxyl end codon by codon by PCR. We overexpressed and purified 15 recombinant proteins of different sizes (571 to 557) to find the more stable protein and thus, the resolution of the crystals. The purification was performed by IMAC and gel filtration techniques.

From the 15 different recombinant fragments, there was only one that induced crystals under the same conditions as our initial fragment (572 aa), the fragment 1-571. The crystals were optimized, and we obtained diffraction images with a resolution of 5 Å. The resolution is not enough to solve a structure, so we are now trying to improve the resolution by changing the crystallization ions, buffers,different concentrations of protein and other methodologies related to the induction and crystal growth.

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Mitochondrial permeability transition is involved in silica nanoparticle-induces cardiac injury through oxidative stress.

Christian Silva-Platas[1], Omar Lozano1, Baruc E. Pérez[1], Héctor Chapoy-Villanueva[1], Yuriana Oropeza-Almazán[1], Alejandro Torres-Quintanilla[1] and Gerardo García-Rivas[1,2,] *.

[1]Cátedra de Cardiología y Medicina Vascular, Escuela Nacional de Medicina y Ciencias de la Salud, Tecnológico de Monterrey.
[2]Centro de Investigación Biomédica, Hospital Zambrano-Hellion, Tecnológico de Monterrey.
* gdejesus@itesm.mx

Dirección: Monterrey, N.L., México 64710. Tél: (81)-8888-0472.

Silica nanoparticles (SNP) become known as promising systems that can deliver biological active compounds to the heart and other organs in a controllable manner. However, cardiac toxicity induced by SNP related to calcium handling, oxidative stress and energetic failure are described. Moreover, little is known about the precise mechanism undelaying this cardiotoxicity. To further investigate on the mechanisms of SNP induce mitochondrial dysfunction, here we explored the *ex vivo* function and subcellular characterization of the heart after exposure to SNP. The cumulative administration of SNP reduced the contractility of the rat heart with a half maximal inhibitory concentration of 23 ppm. Heart rate diminished (316±15 vs 144±21 bpm) and contracture was evident as perfusion progressed. ATP content was reduced and the necrosis markers increased in a time-dependent manner. Electron microscopy revealed the SNP-internalization and the subcellular localization within mitochondria. Isolated mitochondria exposed to SNP showed that the respiratory activity was inhibited in both phosphorylating and non-phosphorylating states. The simultaneous recording of the mitochondrial membrane potential revealed that the SNP depolarize as the dose of 10 ppm is reached, concomitantly, permeability transition pore opening was observed. This depolarization was sensitive to Cyslosporine A and MitoTempo. Moreover, the reduction of relevant thiol groups for the opening of mitochondrial permeability was associated with oxidative damage triggered by SNP. Remarkably, Mitotempo treatment on cardiac cells decreased SNP-induces mitochondrial dysfunction and cell death. In conclusion, the progressive exposure of the heart to SNP may result in the mitochondrial accumulation that depolarize the organelle, inhibit ATP synthesis that produce the energetic imbalance and reduce contractility, triggering cell death.

NMR-based metabolomic analysis on the seasonal variation of *Ternstroemia pringlei* (Rose) Standl.

Alexis Uriel Soto- Díaz[1], Jan Schripsema[2], María Luisa Villarreal[1], Verónica Rodríguez- López[3] Alexandre Cardoso –Taketa[1].

[1]Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos, Avenida Universidad 1001, Colonia Chamilpa, Cuernavaca, Morelos 62210, México.[2]Centro de Ciencias Exactas y Tecnología, Universidad Estatal del Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, Rio de Janeiro, Brasil. [3]Facultad de Farmacia, Universidad Autónoma del Estado de Morelos.

Key words: *Ternstroemia,* metabolomic, ^{1}H NMR.

INTRODUCCIÓN

Ternstroemia pringlei (Rose) Standl. (Theaceae) is a native tree widely used and commercialized in México. The leaves are used in traditional medicine to reduce injuries and rheumatic pain. Previous studies on this plant have demonstrated the presence of glycosylated phenylethanoid compounds with antioxidant and possibly anti-inflammatory activity[1]. However, the spatial and temporal distribution of these compounds in the plant is unknown.

^{1}H Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy and multivariate data analysis have recently been used to obtain the metabolic profile from plant crude extracts[2]. This approach allows to analyze complex matrix as plant extracts are, in order to cluster them in terms of their metabolite content.

A seasonal variation analysis using ^{1}H NMR and multivariate data analysis allows to define the spatial and temporal distribution of certain compounds in the plant. The aim of the present study was to obtain the metabolomic profile of *T. pringlei* individuals and to determine the variation during one year of the metabolites of interest present in their leaves.

MATERIALS AND METHODS

Monthly collections (December-September) of *T. pringlei* leaves (n = 4) were done in the municipality of Huitzilac, Morelos. The methanolic-aqueous extracts were obtained and processed in an NMR equipment (Bruker 500 MHz). ^{1}H NMR records of all samples were integrated and analyzed through the SpinWorks and SIMCA-P (PCA) softwares.

RESULTS AND DISCUSSION

The metabolic profile of *T. pringlei* collected in different months showed significant variations in the presence and concentrations ofmetabolites. Several important compounds were identified (Fig. 1).

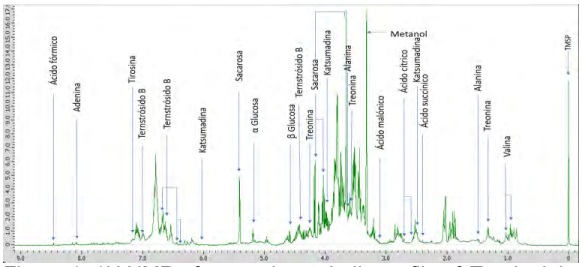

Figure 1. 1H NMR of general metabolic profile of *T. pringlei.*

A principal component analysis was carried out, showing a correlation between the presence of aromatic compounds (ternstroside B and katsumandin) and the temporal factor.

CONCLUSIONS

Multivariate data analysis using 1H NMR allowed to observe a broad panorama of variations in the metabolic content of *T. pringlei*. It was also found that the months of June and August were the most favorable for the production of glycosylated phenylethanoids in the leaves of *T. pringlei*.

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Effect and study of inhibitors of the enzymes DNA topoisomerases I and II on *Candida* spp.

Tagle-Olmedo Tania[1], Andrade-Pavón Dulce[1], Lourdes-Villa Tanaca[1], César Hugo-Hernández Rodríguez[1], Gómez-García O[2].

[1]Laboratorio de Biología Molecular de Bacterias y Levaduras,[2]Laboratorio de Síntesis de Fármacos Heterocíclicos, Escuela Nacional de Ciencias Biológicas del Instituto Politécnico Nacional, Plan de Ayala y Prol. Carpio, Col. Casco de Santo Tomás, México D. F. CP 11340, Phone (+52 55) 57 29 63 00 Ext. 62373. e-mail: tania.tagle@hotmail.com.

Key words: DNA topoisomerases, fungi, bioinformatics, phylogeny, modeling, molecular docking.

The DNA topoisomerasas (Topo I and Topo II) are enzymes that act on the topology of DNA, allowing it to be stored more compactly, in addition to participating in the control of protein synthesis. These enzymes are present in eukaryotic and prokaryotic organisms. Given that they are enzymes that have been proposed as targets for anticancer drugs, we do not rule out the possibility of using the topoisomerases of yeast of medical interest as possible therapeutic targets for the treatment of opportunistic fungal infections. Mycosis caused by yeasts of the genus Candida has been increasing, so the study of these enzymes will allow us to propose them as molecular targets in the future. In order to learn more about the secondary and tertiary structure of this protein and at the same time to predict the possible biological activity, a biochemical and bioinformatic analysis of the amino acid sequences of DNA topoisomerases of ascomycetes and basidiomycete fungi was carried out, as well as a phylogenetic analysis and a molecular modeling-docking study of each of the DNA topoisomerases studied. To this end, studies of inhibition with inhibitory compounds of the DNA topoisomerases were carried out in different clinical isolates of *Candida* spp and DNA topoisomerase sequences were downloaded for their alignment and the topology of each of them was described using the Expasy-Prosite server, a phylogenetic tree was carried out using the MEGA program by the maximum likelihood method, the prediction of the tertiary structures of the DNA topoisomerasas by means of the homology technique were generated for the realization of a molecular docking study between such enzymes and inhibitory compounds of DNA topoisomerases. Inhibition studies revealed that inhibitors of Topo I and II inhibit the growth and viability of *Candida* spp. The alignment and topology of the DNA topoisomerasas revealed that there are highly conserved domains among them, in addition the phylogenetic analysis indicated that the DNA topoisomerasas of basidiomycete

fungi are grouped with the human DNA mole, while those of the species of the *Candida* genus within the group of the ascomycetes, this suggests the use of DNA topo of basidiomycetes as models to study anticancer drugs and that of the ascomycetes for antifungals. The tertiary structure of all DNA topoisomerases is structurally conserved and the molecular docking study with the known inhibitors of these enzymes showed a possible antifungal biological activity. These results suggest the possible use of these enzymes as molecular targets for the search of drugs with anticancer and antifungal activity.

ARP2; a determinant molecule in apoptosis of tumoral cells, new implications in atherosclerosis

Juana Virginia Tapia-Vieyra, Jaime Mas-Oliva. Instituto de Fisiología Celular, UNAM. Circuito Exterior. Cd Universitaria.Tel: 52 55 6 22 55 84. jmas@ifc.unam.mx

Cancer is a multifactorial disease and a major health problem worldwide. Neoplastic epithelial cells can produce the most aggressive types of cancers, such as those that occur in the ovary, lung, colon, prostate and breast. Hormone-dependent tumors such as prostate cancer develop hormonal independence to continue their development as well as a phenotype resistant to apoptosis, so in these advanced stages the progress towards metastasis is very frequent. The therapies that have been used for the treatment of prostate cancer so far have not allowed to control the progress of the disease, hence the enormous need for new therapeutic strategies to stop their development. It is well known that the mechanism of apoptosis when inhibited is a determining factor for the cancer to advance. In several cell types, increases in Ca^{2+} above basal levels promote apoptosis, which is why in our group the proteins that promote these increases and induce cells to programmed cell death have been studied. In androgen-independent lymphoid nodule prostate cancer cells (LNCaP) maintained without serum and under treatment with ionomycin as inducers of apoptosis, a non-selective 23pS cation channel of conductance was identified which promotes incoming Ca2+ currents and the development of apoptosis (1). Subsequently, continuing with this line of research, we carried out the cloning and characterization of the *arp*2 cDNA, whose mRNA was injected into a heterologous expression system of *Xenopus laevis* ovocytes, promoting the activation of Ca^{2+} currents and apoptosis (2). The gene encoding the ARP2 protein was overexpressed in LNCaP cells and Chinese hamster ovary (CHO) cells, showing that ARP2 is initially localized in the perinuclear region and over time migrates to the plasma membrane region, inducing apoptosis (3). The ARP2 protein was overexpressed in human embryonic kidney HEK cells and epithelial cells of endothelial cardiovascular furthermore smooth muscle, promoting apoptosis, which was demonstrated by the activation of effector caspases 3 and 7, and by the TUNEL technique. All evidence so far suggests that ARP2 promotes the incoming Ca^{2+} currents and the increased concentration of this ion in the cytoplasm of epithelial cancer cells, induces apoptosis in these cells whose phenotype shows resistance to programmed cell death, and can additionally contribute to the development of apoptosis when it is overexpressed in the cell types that participate in the atherogenic processes, eventually it could constitute an important therapeutic tool in the treatment of both diseases (4).

Key words: apoptosis, ARP2, epithelial carcinoma, prostate cancer

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Does the NaStEP-NaSIPP interaction in mitochondria trigger programmed cell death in Nicotiana pollen tubes?

Edgar Nájera Torres[1], Yuridia Cruz-González Zamora[1], Javier Andrés Juárez Díaz[2] and Felipe Cruz García[1]
1.- Departamento de Bioquímica, Conjunto E, Facultad de Química, Ciudad Universitaria, México cp.04510.
2.- Departamento de Biología Comparada, Facultad de Ciencias, UNAM. Ciudad Universitaria, Cd. Mx. 04510.
e-mail: fcg@unam.mx

Self-incompatibility (SI) is a genetic-biochemical system that is defined as the inability of a fertile hermaphroditic plant to generate zygotes after self-pollination. SI is controlled by the polymorphic S locus, which encodes and expresses both the female determinant (S-RNase) in the pistil and the male determinant (SLF) in the pollen grain. The interaction between concomitant allelic SLF and S-RNase in the pollen tube activates the pollen rejection mechanism.

Other genes for pollen rejection found outside the S locus, named modifier genes (MG) are essentials to perform the pollen rejection response correctly. So far, four MGs have identified in Nicotiana are HT-B, 120K, NaStEP, and NaSIPP.

NaStEP (N. alata Stigma Expressed Protein), is a protein with protease inhibitor activity and it is express in stigmas of SI Nicotiana species. Suppression of this gene by RNAi in Nicotiana spp. causes break down of SI. NaStEP interacts with NaSIPP (Nicotiana alata Self-Incompatibility Pollen Protein), which is a protein with mitochondrial phosphate transporter activity and is essential for the SI mechanism. It suggests that this interaction may destabilize the membrane potential of the mitochondria and therefore, trigger programmed cell death (PCD).

To test this, we transformed the Saccharomyces cerevisiae Δmir1 with NaSIPP to have a strain with this gene integrated into its genome and then, to transform it with a plasmid to over-express NaStEP, to challenge it with different stimuli that trigger PCD, to demonstrate that NaSIPP-NaStEP interaction initiates PCD. Also, we are evaluating if NaStEP has BH domains, which play a role in cell death.

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Characterization of super-complex with NADP$^+$-isocitrate dehydrogenase activity in mitochondria from human placenta

Viviana Urban-Sosa, Sofía Olvera-Sánchez, Oscar Flores-Herrera, Federico Martínez.
Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México.
Circuito Escolar, Cd. Universitaria. UNAM 04510, México, CDMX. Tel. 5623-2168
fedem@bq.unam.mx

Las mitocondrias del sinciciotrofoblasto de la placenta humana sintetizan progesterona (P4) y ATP. La P4 es esencial para mantener la relación entre el feto y la madre, mientras que el ATP sustenta los mecanismos de transporte de nutrientes de la madre hacia el feto. Se ha reportado que tanto la síntesis de P4 como la de ATP son dependientes del poder reductor mitocondrial (*i.e* NADPH y NADH, respectivamente). Se sabe que el poder reductor en la mitocondria proviene principalmente del ciclo de Krebs y en menor medida de la β-oxidación. Hemos reportado que en las mitocondrias aisladas del sinciciotrofoblasto el metabolismo esteroidogénico es sustentado por el isocitrato [1], mientras que el succinato mantiene el metabolismo energético.

De manera interesante, la presencia de NADP$^+$ e isocitrato promueven la síntesis de P4 en los sitios de contacto esteroidogénicos del sinciciotrofoblasto.

Lo sugiere que la actividad de las coenzimas reducidas, isocitrato deshidrogenasa dependiente de NADP$^+$ (IDH-NADP) o NAD$^+$ (IDH-NAD) tienen repercusión en la síntesis de P4 o ATP., en este trabajo se caracterizó la actividad de la isocitrato deshidrogenasa (IDH) dependiente de NADP$^+$ o NAD$^+$ en mitocondrias aisladas del citotrofoblasto y del sinciciotrofoblasto humano.

En el sinciciotrofoblasto la actividad de la IDH-NADP$^+$ fue de 4.1 x 10^{-2} μmol NADPH/min/mg, y en el citotrofoblasto de 5.1 x 10^{-2} μmol NADPH/min/mg. La adición de calcio (XX μM) no tuvo efecto sobre la producción de NADPH.

Por otra parte, la actividad de la IDH-NAD$^+$ tanto del sinciciotrofoblasto como del citotrofoblasto fue de 6.4 x 10^{-3} μmol NADH/min/mg en presencia de calcio. La ausencia del catión provocó la disminución de la actividad de la enzima en el citotrofoblasto, la cual presentó una actividad de 1.9 x 10^{-4} μmol NADH/min/mg. Como se puede observar la actividad de la IDH-NADP$^+$ es de alrededor seis veces mayor que la actividad de la IDH-NAD$^+$ y es insensible al calcio.

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Biochemical, biophysical and structural characterization of isoniazid resistance KatG variants from *Mycobacterium tuberculosis*.

Brenda Georgina Uribe Vázquez, Humberto Flores Soto and Francisco Xavier Soberón Mainero, Cellular Engineering and Biocatalysis Department, Biotechnology Institute, UNAM, Cuernavaca, C.P. 62210, soberon@ibt.unam.mx

Introduction. Tuberculosis (TB) is an infectious disease caused by the pathogenic agent *Mycobacterium tuberculosis*. Isoniazid (INH), the compound most used for the treatment of TB worldwide, is a pro-drug activated by the catalase-peroxidase (KatG) of *M. tuberculosis* converted into a radical that binds to NAD^+, forming the INH-NAD adduct, which acts as a potent inhibitor of the InhA enzyme, involved in the cell wall biosynthesis. INH-resistance is the result of the accumulation of mutations that alter the function of the KatG protein (~ 70% of resistant isolates have the S315T mutation). (1)
The objective of this study is the characterization of KatG variants, found in INH-resistance samples.

Methodology. The genes of selected variants (S315T, A256T, E287K, L333V and A556S) were constructed by site-directed mutagenesis with the addition of 6 histidine codons in the 5' of the gene and were cloned in the expression vector pKK-223-3. The proteins were purified by affinity column Ni-NTA. The catalase, peroxidase and adduct formation (INH-NAD) activities were measured. (1)

Results. Plasmid pKK-KatG allowed high levels expression of soluble recombinant proteins. Enzymatic assays confirmed that the variants are functional. However, they diminish the adduct formation compared with the KatG-WT; this is according to the observed phenotype resistance (the KatG-L333V variant is an exception).

Conclusions. A KatG expression system in *E. coli* was constructed. The mutant S315T produces less adduct than the WT, which correlates with that reported in the literature. The variants KatG-A256T, -E287K and -A556S have lower activity of adduct formation compared to the WT, which correlates with the reported resistance.

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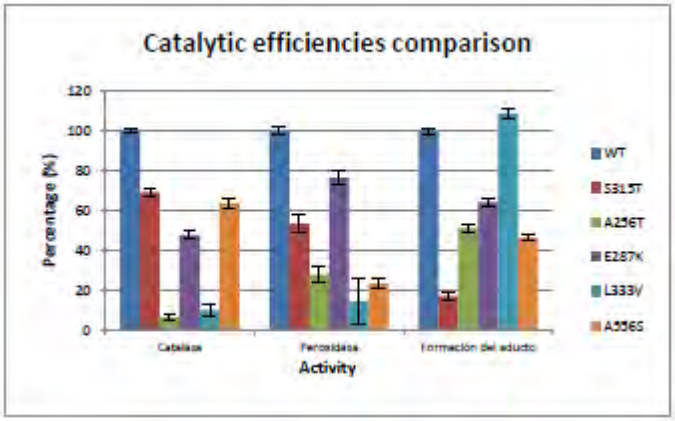

Graph 1. Catalytic efficiencies comparison of the variants associated with resistance, assuming the KatG-WT activities as 100%.

Cis-regulatory evolution leads to the expression of the *phoH* ancestral gene in conditions relevant for *Salmonella* virulence

Marcos Antonio Valdespino Díaz[1] and Víctor Humberto Bustamante Santillán[1*]

[1]Instituto de Biotecnología, UNAM.
Av. Universidad #2001, Col. Chamilpa C.P. 62210, Cuernavaca, Morelos. Phones: (52 777) 329 16 00, (52 55) 56 22 76 00. *email: victor@ibt.unam.mx

Introduction. *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) causes severe enteritis in humans and various animals, as well as systemic infection in laboratory's mice, chickens and immunocompromised humans. Most of the genes required for the *S.* Typhimurium invasion of host cells are in the *Salmonella* Pathogenicity Island 1 (SPI-1), a chromosomal region consisting of 39 genes. The AraC-like transcriptional regulator HilD, encoded in SPI-1, induces the expression of the SPI-1 genes and several other virulence genes located outside SPI-1, when *Salmonella* is grown in the nutrient-rich lysogeny broth (LB). In a recent study, we determined that HilD directly and positively regulates the expression of the *phoH* gene, when *S.* Typhimurium is grown in LB. *phoH* is an ancestral gene present in many bacteria, including *Escherichia coli* K-12 and *Salmonella bongori* (a species evolutionarily between *E. coli* and *S.* Typhimurium). The PhoH protein has ATP binding activity; however, its cellular function remains unknown. In *E. coli* K-12, *phoH* expression is induced by the PhoR/PhoB two-component system, in response to phosphate starvation. Since the regulation by HilD represents a novel mechanism for the control of *phoH* expression, in this study, we analyzed the regulatory evolution for *phoH*, from *E. coli* K-12 to *S*. Typhimurium.

Results. We determined the expression of *phoH* from *S*. Typhimurium, *S. bongori* and *E. coli* K-12 in each of these three bacteria, through transcriptional fusions to the *cat* reporter gene. Interestingly, *S*. Typhiumrium and *S. bongori phoH*, but not *E. coli phoH,* were expressed in LB, in a HilD-dependent way. Our results indicate that HilD directly activates the promoter 1 of the *S.* Typhimurium *phoH*. Surprisingly, the expression of *E. coli phoH*, but not that of *S*. Typhimurium *phoH,* was regulated by the PhoR/PhoB system, in response to phosphate starvation. Consistently with these results, we found that the sequence upstream of *phoH* is highly conserved between *S.* Typhimurium and *S. bongori*, but it is poorly conserved between *Salmonella* and *E. coli* K-12. Together, our results indicate that changes in the upstream regulatory sequence of *phoH*, during the divergence of *Salmonella* from *E. coli* K-12, generated the HilD-mediated regulatory connection that induces the expression of *phoH* in conditions relevant for *Salmonella* virulence.

Biochemical and physiological characterization of plasma membrane H+-ATPases PMA1 and PMA2 from corn smut basidiomycete *Ustilago maydis*

Melissa Vázquez-Carrada[1,2], Minerva Georgina Araiza-Villanueva[1,2], Rodrigo Prieto-Carrasco[1,2], Juan Pablo Pardo[2], Guadalupe Guerra-Sánchez[1]

[1] Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional. Prolongación de Carpio y Plan de Ayala s/n, Santo Tomás, Miguel Hidalgo, 11340, Ciudad de México, México. 5729600 ext. 62339, lupegs@hotmail.com
[2] Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México. Ciudad Universitaria, Apartado Postal 70-159, Coyoacán, 04510, Ciudad de México, México. 56232175 pardov@bq.unam.mx

Abstract

Plasma membrane H^+-ATPases of fungi, yeast and plants act as proton pumps to generate a proton electrochemical gradient essential for nutrients secondary transport and intracellular pH maintenance. *Saccharomyces cerevisiae* has two genes (*pma1* and *pma2*) that coded H^+-ATPases. The *pma1* gene is the predominant one in the fungal physiological functions. Plants have a larger number of genes that code for proteins with structural and functional similarity to the previous ones. *Ustilago maydis* is a biotrophic basiodiomycete that infects corn and teozintle. In this dimorphic fungus, which transits from a non-infective haploid phase to a diploid phytopathogenic mycelium, the presence of two H^+-ATPase-encoding genes has been described, one with high identity with that present in fungi UmPMA1 (*um02851*) and the other similar to that of plants UmPMA2 (*um01205*). Unlike *S. cerevisiae*, these two genes are expressed jointly in *U. maydis* yeast. In the present work, mutants lacking these genes (ΔPMA1 and ΔPMA2) were used to investigate the role of each of the H^+-ATPases in the haploid and mycelial phase of the fungus. To approach this, microbiological studies were carried out, as well biochemical (enzymatic activity, glucose and oxygen consumption, proton pumping rate, membrane potential) and microscopy assays. The results indicated that both H^+-ATPases work jointly for the membrane potential maintenance and the electrochemical proton gradient generation useful for secondary transport.

Analysis of the control of the trypanothione synthesis in *Trypanosoma cruzi*

Citlali Vázquez., Zabdi Gónzalez-Chávez, Marlen Mejia-Tlachi, Emma Saavedra. Instituto Nacional de Cardiología Ignacio Chávez, 14080, Ciudad de México. emma_saavedra@hotmail.com, tel: 55732911 ext. 25500

American trypanosomiasis is caused by the protist parasite *Trypanosoma cruzi*. The current treatment for the infection use benznidazole despite its toxicity and low efficacy for the infection in human adults. Therefore, there is a need of safer drugs and drug targets to establish new therapeutic strategies. In this regard, trypanothione ($T(SH)_2$), a conjugate of two glutathione (GSH) and a spermidine molecule is the main antioxidant metabolite in *T. cruzi*, replacing the antioxidant functions of GSH in mammalian cells. However, the enzymes involved in $T(SH)_2$ synthesis need to be validated as drug targets. Through computational metabolic modeling it was predicted that gamma-glutamylcysteine synthetase (ECS) and trypanothione synthetase (TryS) control by 58 % and 49 %, respectively, the flux of $T(SH)_2$[1].

T. cruzi epimastigotes overexpressing different levels of ECS (OE-ECS) or TryS (OE-TryS) were obtained and their effect on thiol contents and pathway flux were monitored. Increased activities of 3-7 folds and 20-30 folds were attained in the OE-ECS and OE-TryS clones, respectively. OE-ECS parasites showed up to 4-fold increase in $T(SH)^2$ but OE-TryS did not. Later, the transfectants were supplemented with 0.1 mM Cys or 1 mM GSH; OE-ECS synthesized 6 times more $T(SH)_2$ vs control parasites, whereas OE-TryS increased the thiols similar to the control cells. It was further found that the high TryS expression promoted $T(SH)_2$ degradation, which explained the lack of increase in $T(SH)_2$ despite the high TryS activity. Indeed, the levels of malondialdehyde were the highest in the OE-TryS parasites.

Further, the $T(SH)_2$ synthesis fluxes were determined in control cell and OE-gECS clones, attaining values of 0.6 mU/mg cell protein and up to a value of 2.4 mU/mg cell protein, respectively. Afterwards, the flux control coefficients (C^J_{ai}) of ECS was determined. The C^J_{ai} is a quantitative value of the degree of influence that an enzyme has in setting the pathway flux; an enzyme with a C^J_{ai} approaching to one, means that has total control of the pathway flux 2. From the plots of percentage of flux *versus* percentage of enzyme activity, the C^J_{ai} is calculated from the derivative at the wild type level of enzyme activity. A C^J_{ai} of 0.69 ± 0.15 for ECS on $T(SH)_2$ synthesis was determined, which is in agreement with the control predicted by metabolic modeling. It is also predicted that TryS has a C^J_{ai} of at most 0.3.

It is concluded that ECS is the main controlling enzyme of T(SH)2 synthesis in *T. cruzi* and its inhibition will greater affect the parasite antioxidant defense than inhibition of low-controlling enzymes. Although, TryS has lower control it is absent in human cells and could be also an adequate drug target but it will require higher inhibition levels.

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Biochemical characterization of the thermolabile lecithin dependent hemolysin of *Vibrio parahaemolyticus*

Luis Enrique Vazquez Morado [a]. Ramón Enrique Robles Zepeda [a]. Adriana Garibay Escobar [a]. Aldo Alejandro Arvizu Flores [a], Al onso Alexis López Zavala [a], *
[a] Universidad de Sonora, Hermosillo, Sonora Campus Hermosillo, C.P. 83000, México. * Project director, e-mail: alexis.lopez@unison.mx, phone: (662)172 2240.

Thermolabile lecithin dependent hemolysin (*LDH*) is a virulence factor of *Vibrio parahaemolyticus* (*Vp*) of 47.3 KDa that acts as phospholipase A2 with lysophospholipase activity which produces hemolysis and cell damage. *LDH* has been related to the infectious process of *Vp* that causes systemic vibriosis that impacts negatively shrimp culture. Despite of this information remains unknown the role of *LDH* during *Vp* infection in shrimp and its biochemical characteristics which could contribute to establish alternative strategies against shrimp vibriosis. *LDH* was obtained as a recombinant protein in rosetta 2 *E. coli* strain and was purified to electrophoretic homogeneity by immobilized metal affinity chromatography (IMAC) from the inclusion bodies under denaturing conditions (8M urea). Then, denatured *LDH* was refolded by dialysis decreasing the urea concentration to 0 M. Enzymatic activity of *LDH* was evaluated by UV/VIS spectroscopy using p-nitrophenyl laurate as a substrate in presence of 0.0001 % of egg yolk lecithin (EYL). Also, phospholipase activity was assayed in 1.5% agar plates containing 0.1% of EYL. Additionally, human erythrocytes were used to evaluate *LDH* hemolytic activity. Both optimal temperature and pH was evaluated as well as thermal stability. The *LDH* was eluted as a single peak by IMAC at 23% imidazole, then *LDH* was refolded to its active form which was confirmed by a single band in 12 % SDS-PAGE and solid-state phospholipase assay. *LDH* is obtained at a ratio of 3.5 mg. of enzyme per gram of bacterial pellet. *LDH* was able to hydrolyze p-nitrophenyl laurate with 7.3 units/mg of protein and shows hemolytic activity of 76.18 % compared to tween 20 (100 %) against human erythrocytes. *LDH* shows high enzymatic activity at optimal pH=8 and the apparent optimum temperature was 50°C by 5 minutes. LDH was incubated by 15 minutes with a temperature range of 10-80 °C, the enzyme retains 80% of residual activity at 40°C. However, no enzymatic activity was recorded above 60ºC. Thermal stability data were fitted to non-linear Boltzmann equation model (R2= 0.9945) finding a Tm=50.94°C. Initial velocities were analyzed using Arrhenius plot achieving to calculate activation energy (Ea=-28,725.90 J/m at 90 seconds of reaction) indicating that the enzyme is suddenly denatured., These results indicates that *LDH* is a thermolabile hemolysin compared with both –*thermo direct and thermo related* hemolysins from Vp.

Integrity, activity and stoichiometry of the respiratory supercomplexes of *Ustilago maydis*.

Héctor Vázquez-Meza, Deyamira Matuz-Mares, Juan Pablo Pardo. C.P. 04510, Tel: 56232168, Fax: 56162419; pardov@bq.unam.mx. Department of Biochemistry, Faculty of Medicine, UNAM, Av. Universidad 3000.

Ustilago maydis is a phytopathogenic fungus that infects the corn plant (*zea mays*) generating what is commonly known as huitlacoche. This basidiomycete has been used as a model to plant-pathogen interactions and a large number of in biotechnology applications. The mitochondrial respiratory chain of *U maydis*, in addition to presenting the four classical respiratory complexes (complexes I, II, III, and IV) and the ATP synthase, has others components (NADH dehydrogenase or alternative oxidase [Aox1]) that might important to the fungus at the time of invading its host.

In our laboratory we are interested in characterizing the *U maydis* mitochondrial respiratory chain under different growth conditions, in addition to carrying out proteomic studies to determine if the accessory or alternate proteins are essential components of the respiratory supercomplexes.

Results: when the fungus grows in a rich medium (YPD), the stationary phase of the growth curve is reached 24 hours after the start of the crop. When using a minimum medium with glucose or ethanol as a carbon source, the growth curve is similar to that found with YPD; however, if we use lactate or glycerol as carbon sources, the growth is very slow and the stationary phase is reached around 150 hr (Cardenas-Monroy *et al*., 2017). On the other hand, the integrity and good condition of the isolated mitochondria of *U maydis* were determined by their respiratory control (RC), using the method reported by Sierra-Campos *et al*., (2009). In each of the experimental conditions tested, we found the same respiratory supercomplexes, but the proportion of these varied, comparing the YPD medium with respect to the minimum media with the different carbon sources. Which makes us suppose that the carbon source is important for the integration and activity of supercomplexes.

The proteomic analysis indicates that the alternative oxidase (Aox1) of *U maydis* is present in all the bands containing complex I suggesting that Aox1 in important component of the respiratory supercomplexes of the fungus. Alternative NADH DH was not associated to supercomplexes

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Structural stability of the glucose-6-phosphate dehydrogenase of *Pseudomonas aeruginosa* provided by its substrate

Roberto Velasco-García[1], Edaena Benítez-Rangel[1], Annia Rodríguez-Hernández[2] y Adela Rodríguez-Romero[2].

Facultad de Estudios Superiores Iztacala1 e Instituto de Química2, UNAM. C. P. 52970. Tel. 56 23 12 60. robertvela2001@yahoo.com.mx

P. aeruginosa is a pathogen of clinical relevance because it is the causal agent of numerous intrahospital infections. Because of this, the search for new therapeutic targets in the microorganism is of vital importance. An essential molecule for *P. aeruginosa*, and thus a potential target for antipseudomonal agents, is the enzyme glucose-6-phosphate dehydrogenase (G6PDHPa), which participates in the EDEMP cycle. This cycle is formed by enzymes of the Entner-Doudoroff (ED), Embden-Meyerhof-Parnas (EMP), and pentose phosphate pathways (PPP). Specifically, G6PDHPa, as the enzyme of other organisms that also possess the EDEMP cycle, oxidizes glucose-6-P (G6P) to 6-P-glucono-δ-lactone, with the concomitant production of NADPH or NADH. Conversely, the G6PDH of eukaryotes, which catalyze the same reaction in the PPP, seem to be specialized in the production of NADPH, since they have negligible or no activity with NAD+. Several studies indicate that the increase in the concentration of NADP+ stabilizes the human enzyme and other eukaryotic G6PDHs. In the present study, we demonstrated, through the monitoring of the residual enzyme activity by spectrophotometry at a wavelength of 340 nm, that the G6P, but not NADP+, increases the stability of the recombinant G6PDHPa and protects it from thermal inactivation and trypsin proteolysis. Fluorescence studies, following the emission of the ANS fluorescent probe between 400 and 600 nm, suggest that this stability is due to conformational changes in the protein produced by the cooperative binding of the substrate to the enzyme (nH = 1.53 ± 0.08). The K_{dapp} estimated for the G6P-G6PDHPa complex was 74.7 ± 2.5 µM. The change in the enzyme conformation, achieved with a saturating concentration of G6P (6.0 mM), presented two phases, with apparent first-order rate constants of 66 ± 1.6 y 0.21 ± 0.002x10^{-2} min^{-1}, for the fast and slow phases, respectively, and amplitude of the fast phase of 76%. The modified enzyme showed higher substrate affinity than the native enzyme, while the analysis of molecular mass by size exclusion chromatography indicated that the native enzyme is a tetramer (220 kDa) and that modified by the substrate has a greater molecular mass and reaches equimolar equilibrium with the tetramer, after incubation with G6P. These findings demonstrate mechanistic and conformational differences between G6PDHPa and those enzymes from eukaryotic organisms, which could reflect different regulatory mechanisms *in vivo*.

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Layered Double Hydroxides (LDH) for the immobilization by metal affinity of genetic engineered enzymes for the development of biosensors.

Gilberto Velázquez-Juárez, Adrián Venegas Reynoso, Gregorio Carbajal Arizaga.
Universidad de Guadalajara. Centro Universitario de Ciencias Exactas e Ingenierías. Departamento de Química.
Blvd. Marcelino García Barragán #1421, esq Calzada Olímpica, C.P. 44430, Guadalajara, Jalisco, México. Teléfono:+52 (33) 1378 5900 EXT 27682. email: gilvelazquez83@gmail.com

ABSTRACT

Searching for a simple and reliable scheme to immobilize enzymes is of great importance.The strong affinity link between a metal cation and a chelator such as nitrilotriacetic acid (NTA) or imidodiacetic acid (IDA) has been used to develop enzymatic biosensors, nevertheless these materials are registered or patented1, becoming difficult its use for commercial purposes.

Development of new materials for enzymatic affinity immobilization overcomes that impediment. Layered double hydroxides (LDHs), are a great option for making new materials2,they can be expressed with the following general formula: $[M^{II}_{1-x}M^{III}_{x}(OH)_2]\times[A^{n-}_{x/n}\times mH_2O]$.

Where, M^{II}_{1-x} are divalent cations (Mg^{2+}, Cu^{2+},Zn^{2+}, Co^{2+}, Ni^{2+}); M^{III}_{x} are trivalent cations (Al^{3+}, Cr^{3+},Fe^{3+}), and A^{n-} is an interlayer anion (Cl^-, NO_3^-, CO_3^{2-}, SO_4^{2-}). In this work we have developed a new LDHs with Ni^{++}, Cu^{++} and Co^{++} atoms incrusted. We have chosen those metals because they're well known to coordinate molecules. LDHs were produce by alkaline precipitation and confirmed by spectroscopic technics (XRD and IR). After materials were obtained, we decided to prove if their were capable of immobilize proteins. In order to do that, we produced an engineered 6xhis tag cathecol dioxygenase[3] enzyme from *Arthrobacter sp.* Protein was obtained by synthetic biology methods and overexpressed at *E. coli* strain. After several purification steps (nickel affinity and ionic exchange), we confront our purified enzyme with aforementioned HDLs. We found that our materials exhibit strong immobilization for the protein of interest (visualized by SDS PAGE). Immoblization was able to resist the presence of competitors such salts (1M),detergents (1%) and imidazole (1000 mM). These results suggest that our materials could be use for development of enzymatic biosensors.

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Key words: HDL; immobilization; genetic engineering; biosensors.

PGC-1 and perilipin during physiological cardiac hypertrophy induced by pregnancy

Denisse Verduzco-Ávila[1], Diana Lourdes Padilla-Bórquez[1], Susana Castro-Meza[1], José Arquímides Godoy-Lugo[2], Rudy Ortiz[2], José Guadalupe Soñanez-Organis[1*].

[1]Universidad de Sonora, Depto. de Ciencias Químico-Biológicas y Agropecuarias, Universidad de Sonora Unidad Regional Sur, Apartado Postal 85880, Navojoa, Sonora, México.
[2]School of Natural Sciences, University of California Merced, 5200 N Lake Road, Merced, CA 95343, USA.
*Corresponding author: Dr. Jose Guadalupe Soñanez-Organis Depto. de Ciencias Químico Biológicas y Agropecuarias Universidad de Sonora Unidad Regional Sur Apartado Postal 85880, Navojoa, Sonora, México Email: jorganis@navojoa.uson.mx or josegpeso@gmail.com

ABSTRACT

Peroxisome proliferator-activated receptor γ (PPARγ) and peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α) are coordinately up-regulated in hypertrophic cardiomyopathy and cooperate to mediate changes in lipid metabolism and storage that are characteristic of and contribute to common forms of heart disease. Previous results in our laboratory suggesting that PPARγ regulate the shift in cardiac metabolism during physiological cardiac hypertrophy. On the other hand, the family of perilipin (Plin: 1-5) are involved in the formation of lipid droplets in various tissues, and it has been demonstrated that Plin5 participates as a protective heart during myocardial ischemia and diabetic cardiomyopathy. However, the participation of PGC-1α and Plin family in physiologic hypertrophy induced by pregnancy and its reversible process (postpartum) are not well defined. Therefore, the goal of this study was evaluated the PGC-1α and Plin family gene expression before, during, and after pregnancy in the left ventricle of rat. Expression of mRNA for PGC-1α increased 18- and 11-fold during pregnancy and postpartum, respectively. Plin1, Plin2 y Plin5 transcript increased 9- to 16-fold and 2- to 11-fold during pregnancy and postpartum, respectively, while expression of Plin3 did not change and Plin4 was not detected. The results demonstrate that pregnancy-induced, physiological cardiac hypertrophy activates the expression of genes involved in lipid storage suggesting that the shift in cardiac metabolism is mediated by the activation of PPARγ/PGC-1α.

A suppressor mutation of Mss51 restores Cox1 synthesis.

Cristian Alberto Vergara Gerónimo, Yolanda Margarita Camacho Villasana, Thomas D. Fox, Xochitl Pérez Martínez.
Departamento de Genética Molecular, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México. Mexico City 04510, Mexico. Tel. +52 55 56225662 email: xperez@ifc.unam.mx

Cox1 is the subunit I of cytochrome *c* oxidase, the terminal oxidase of the mitochondrial electron transport chain. Cox1 is encoded by the mitochondrial gene *COX1* that is expressed through the mitochondrial transcription and translation mechanisms1.

Cox1 synthesis is a highly regulated process aimed to decrease the generation of incomplete, unproductive and pro oxidant species[2]. In yeast cells, this regulation operates at the translation initiation step through the translational activators Mss51, Pet309 and Pet54. These proteins are encoded in the nuclear genome and are post translationally imported into mitochondria, where they act on the *COX1* mRNA 5'-UTR to allow translation initiation[3,4].

Mss51 also acts as a chaperone by transiently interacting with newly synthesized Cox1[5]. Thus, Mss51 is an essential factor in synthesis and proper folding of Cox1[3].

Aiming to understand Mss51 function, in this study we characterized a suppressor mutation of the functions of Mss51 in Cox1 synthesis. We previously determined that it is a nuclear mutation. We found that this suppressor mutation depends specifically on the *COX1* open reading frame to operate. Going further, we demonstrated that this mutation also suppresses the functions of Pet54 in Cox1 synthesis. Because Pet54 is required to recycle Mss51 from the chaperone conformation to the translational activator conformation4, we propose that the nuclear suppressor might promote Mss51 recycling.

Our findings contribute to expand the understanding of the regulation of Cox1 synthesis. This describes a novel nuclear regulator of the Cox1 synthesis.

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Kinetic and thermodynamic characterization of the folding of the lysinearginine-ornithine binding protein (LAO) and its individual domains.

Jesús Renán Vergara Gutiérrez; Tania Berrocal; Eva Mejía; Miguel Costas; Rogelio Rodríguez; Alejandro Sosa; Daniel Alejandro Fernández Velasco.
Laboratorio de Fisicoquímica e Ingeniería de Proteínas, Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad Universitaria. Ciudad Universitaria, 04510, Ciudad de México, México.
Phone number: +52 5556232259. E-mail: renanvg05@gmail.com (JRVG), fdaniel@unam.mx (DAFV)

Proteins carry out a large number of functions in the cell, like catalysis, transport and signaling. All these functions depend on the three-dimensional structure that they adopt in a process known as protein folding. More than fifty year ago it was discovered that the information required for proteins to fold is encoded in the amino acid sequence, however, it is yet not possible to predict the three-dimensional structure that a sequence will adopt (if the sequence is not homologous to another with known structure) this is known as the protein folding problem. Most studies on the mechanism of protein folding have been performed using small (<100 residues) monomeric single domain ~~-m~~odel" proteins. Nevertheless, ~~-a~~verage" proteins are larger (ca. 250 residues) and generally composed of more than one domain and/or subunit. Experimental data on the folding of such proteins is required in order to have a broader picture of folding mechanisms. For this reason, we characterized the thermodynamic and kinetic propertied of the folding process in the lysine-arginineornithine binding protein (LAO), a 238 residues bi-lobular protein; additionally, we studied the folding behavior of the individual domains of LAO (known as A and B) to figure out how this process evolves in going from simple to complex proteins.

Temperature and urea unfolding experiments, followed by spectroscopic techniques,showed that LAO folds in a two state process (the simplest way to fold), at 25 °C and pH=9.0, ΔG = 12 kcal mol^{-1}. However, kinetic experiments turned out to be more complex: unfolding kinetics presented only one rate constants while refolding experiments showed two. As a consequence, the chevron plot has two refolding branches, both of them curved. Double-jump and interrupted refolding experiments showed that this chevron behavior is the result of proline isomerization as well as the presence of an on-pathway intermediate. The equilibrium folding characterization of the individual domains showed two state behavior as well.

Nevertheless, the calculated stabilities were 5.5 kcal mol^{-1} for domain A and just 0.8 kcal mol-1 for domain B. These data indicate that domain B needs to be covalently attached to the domain A in order to be stable and implies a high cooperativity between both domains in the folding of LAO. Moreover, kinetic experiments were more complex for both domains compared to LAO, indicating a more complicated folding mechanism. Based on these results we can conclude, contrary to what we expected, that the mechanism of folding of the isolated domains is much more complex than that of the whole molecule.

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Mitigation of oxidative stress in neurodegenerative processes by the activation of aldehyde dehydrogenases.

Belem Yoval Sánchez, José S. Rodríguez Zavala.
Instituto Nacional de Cardiología "Ignacio Chávez"
Juan Badiano No. 1 Col. Secc. XVI, Delegación Tlalpan 14080, CDMX.
Email: yoval_s@yahoo.com.mx

The incidence of neurodegenerative disorders such as Alzheimer and Parkinson has increased in the last years. Nevertheless, there is a lack of development of drugs to treat these pathologies in the last 50 years. Thus, it is necessary to design protocols to counteract the effects of this type of disorders, which affect approximately 2% of population older than 65 years of age. In these processes, there is a progressive damage to the mitochondrial metabolism, which triggers the generation of reactive oxygen species, which in turns generates lipid peroxidation and accumulation of aldehydes that are highly toxic to the brain. 4-hydroxy-2-nonenal (4-HNE) is one of the most cytotoxic lipid aldehydes, promoting progressive cell loss. In this regard, aldehyde dehydrogenases (ALDHs) catalyze the oxidation of lipid aldehydes and are also involved in the metabolism of 3,4-dihydroxyphenylacetaldehyde (DOPAL), 3,4-dihydroxyphenylglycolaldehyde (DOPEGAL) and succinate semialdehyde, playing an important detoxifying role. These metabolites regulate very important functions in the brain such as learning, memory, movement and behavior. However, at high concentration they become cytotoxic and promote the generation of 4-HNE. Although 4-HNE is substrate of ALDHs, these enzymes are susceptible to inactivation by this aldehyde, which interferes with the biotransformation of catecholamines and neurotransmission, a situation that is linked to the increment in oxidative stress and uncontrolled production of endogenous neurotoxins that are associated with neurodegenerative processes. Therefore, deregulation of the activity of ALDHs, causes accumulation of biogenic aldehydes, neurotoxicity, increased oxidative stress, apoptosis and cell death. Thus, the aim of this work is to modulate the activity or expression of different isoforms of ALDH in a neurodegeneration model and then, propose a therapeutic strategy to contribute to the mitigation of the oxidative stress that is generated in this type of disorders to protect the neurons.

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"Expression of single chain variable fragment scFv 6009F in *Pichia pastoris*"

Mariel Adame[1], Gerardo Corzo[2], Hilda Vásquez[2], Elba Villegas[*1]
Centro de investigación en Biotecnología[1], Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos 62209. Instituto de Biotecnología[2], Universidad Nacional Autónoma de México, Cuernavaca, Morelos 62209. *Corresponding Author: elbav@uaem.mx

Clue words: Humanized antibody, heterlgus expression

The need to generate safer and more efficient antibodies to be used in human therapy has led to the search for strategies such enzymatic digestions or recombinant methods. The manufacturing of antivenoms has benefited of these technologies, resulting in products with more appropriate pharmacokinetic and pharmacodynamic properties, improved specific activity and reduced risks of side effects, including life-threatening anaphylactic reaction. Riaño-Umbarila *et al.,* 2005, reported the generation of a scFv library through RT-PCR, from RNA obtained from B lymphocytes from human peripheral blood, to identify possible anti-Cn2 antibodies (Cn2 toxin of *Centruroides noxius).* The scFv 6009F, whose affinity for Cn2 toxin was 410 pM and was able to neutralize 2 LD50 of Cn2 toxin when injected into mice after being pre-incubated with the toxin in a 1/10 molar ratio, however, scFv 6009F showed an expression yield in *E. coli* TG1 1.1 mg/L. The need to obtain scFv 6009F highest production levels led us to evaluate it´s production in *P. pastoris* strain KM71 using the expression vector pPIC9. Specific oligonucleotides to amplificate the gene scFv 6009 by the polymerase chain reaction (PCR) were designed, in the 5'-end Forward oligo the site for the enzyme EcoRI (GAATTC) was added and for the 3'-end Reverse oligo the site for the AvrII enzyme (CCTAGG) was added, these sites allowed us to insert the scFv 6009F gene into the plasmid pPIC9. *P. pastoris* was transformed by electroporation with purified expression plasmids scFv 6009F/pPIC9, previously linearized with SalI (favoring vector integration at the HIS4 gene locus of the yeast genome and determining the Mut+ phenotype). Transformed colonies were selected on a medium lacking histidine, where only the plasmid-containing cells are able to grow. Transformed *P. pastoris* were grown on MD media for three to five days. Then *P. pastoris* was grown for 144 h in BMGY medium (1% yeast extract, 2% peptone, 100mM potassium phosphate, pH 6.0, 1.34% yeast nitrogen broth, 1% glycerol), and induced in BMMY were glycerol was replaced by using two different methanol concentrations respectively, that is 0.5 and 1.0% at two temperatures 20 and 28 °C. The best expression conditions were 1% methanol at 20 °C. with a yield of 68 mg/L of total protein. When lower temperatures were used fewer endogenous proteins were secreted, which favors the purification processes. The scFv 6009F of ~ 27 kDa was visualized with 12.5% polyacrylamide gels and detected with anti-his tag. Final scFv 6009F antibody concentration and its activiity are been evaluated.

Permutations of the loops of Ts1 by those of the neurotoxin CssII: in vitro folding and specificity on voltage-dependent sodium channels

[1]Germán Obed Aguilar Carlos; [2]Georgina Estrada; [1]Herlinda Clement; [1]Gerardo Corzo

[1]Instituto de Biotecnología, UNAM. *Av. Universidad #2001, Col. Chamilpa C.P. 62210. Tels: (52 777) 329 16 00, (52 55) 56 22 76 00. Cuernavaca, Morelos.* E-mail: corzo@ibt.unam.mx. 2Unidad de Bioquímica y Biología Molecular de Plantas Centro de Investigación Científica de Yucatán A.C. Calle 43 No. 130 Col. Chuburná de Hidalgo. Mérida, Yucatán 97200.

Scorpion toxins that affect voltage-gated sodium channels (Nav) are the most reactive proteins and are the main agents responsible for the neurotoxic effects of scorpion sting poisoning. They recognize the Navs of insects and mammals and produce an anomalous depolarization in excitable cells of the nervous system (Quintero-Hernández *et al*, 2013). The study of scorpion toxins that affect sodium channels have received numerous attention because of their medical importance and because of the interest in characterizing its pharmacological effects at molecular level (Rodríguez De La Vega & Possani, 2005). Likewise, the knowledge on scorpion toxins has been accelerated markedly because of the heterologous systems used for their expression rather than from the isolation from natural sources (Gurevitz & Gueta, 2016). Yet, certain toxins have proven to be difficult to express by recombinant means mainly because of folding problems that do not favor the correct formation of disulfide bridges, resulting in a variety of non-active toxin isoforms. So, one of the most important issues for the heterologous expression of scorpion toxins is to find the correct folding conditions that allow the recombinant molecules to be properly folded and biologically active (Estrada et al., 2007). Therefore, in this study we implemented permutations of the protein loops, between the cystines, of two scorpion toxins, Ts1 and CssII, that have been extensively investigated, generating then chimeric constructions with the nature of such toxins, and expressing Ts1-CssII chimeras in *E. coli*. The expressed chimeras allow us to analyze their folding patterns and to observe how these loop changes affect the activity of such chimeric toxins. Currently, five chimeric mutants were heterologous expressed and folded *in vitro* and their toxicity has been tested in mice model.

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Characterization of extracellular enzymatic extract obtained from *Phlebia floridensis* strain isolated from Yucatan Peninsula with a novel peroxidase and oxidase profile

Roberto Amezquita-Novelo1, Elizabeth Ortiz-Vázquez 1, Denis Magaña-Ortíz1,*
1División de Estudios de Posgrado e Investigación, Instituto Tecnológico de Mérida, Teconlógico Nacional de México, Av. Tecnológico km. 4.5 S/N, C.P. 97118, Mérida, Yucatán, México.
*denis.magana@itmerida.mx

White rot fungi can degrade different types of recalcitrant and xenobiotic compounds present in the environment due to their natural ability to modify chemical bonds of various phenolic components of lignin and their derivatives[1]. Initially, twelve strains of fungi were isolated in the Yucatan Peninsula, only one of them demonstrated degradation on different dyes in preliminary screening; this strain was identified as *Phlebia floridensis* using ITS sequence analysis. In the present work, extracellular enzymatic extract of this strain of *P. floridensis* was studied. More extensive screening assays were performed on agar minimal medium plates using phenolic and non-phenolic compounds, anthroquinone dyes and triphenyl methane dyes. As a result, *P. floridensis* strain was able to mineralize Aniline Blue, Methyl Blue, Brilliant Blue G250, Brilliant Blue R250, Malachite Green and the following substrates: 2,6-dimethoxyphenol (2,6 DMP), N,N-Dimethyl-p-phenylenediamine (DMPPDA), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and Guaiacol (GUA). Thus, *P. floridensis* strain showed extremely versatile profile of enzymatic extract. Subsequently, we determined the presence of proteins from 20 to 70 kDa using SDS-PAGE. After this, cation exchange chromatography was carried out establishing that the fraction with maximum activity was eluted at a concentration of 0.1M NaCl. This fraction was analyzed using different phenolic and non-phenolic compounds. We detected a band of 50 kDa with oxidase activity using guaiacol as substrate. This band was sequenced using HPLC / MS and the spectra obtained were compared with different protein databanks. As a preliminary result we identified this enzyme as a chloroperoxidase. Based on these results, we suggest that this strain could be a source of new peroxidases and useful oxidases in different biotechnological processes.

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Analysis of cellulases produced by microorganisms isolated from sites contaminated by heavy metals

Alondra Andrade González, César Osiris Arias López, Lizbet Alejandra Gutiérrez Chávez, José Antonio Aranda Navarro,Anastasio Cortés Mendoza, Agustín Hilario Rocha Ramírez, *Juan Francisco Sánchez López

Unidad Profesional Interdisciplinaria de Ingeniería campus Guanajuato del Instituto Politécnico Nacional, Departamento de Formación Integral e Institucional. Mineral de Valenciana No. 200, Fracc. Industrial Puerto Interior, C.P. 36275. Silao de la Victoria, Guanajuato, México. Tel. 015557296000 ext. 81380. *Corresponding author: francisco999@hotmail.com

Cellulases are enzymes that bioconvert cellulose into sugars. In the degradation of cellulose, three enzymes are involved: endoglucanases (EC 3.2.1.4), exoglucanases (EC 3.2.1.91) and □-glucosidases (EC 3.2.1.21). In our working group we have isolated and identified fungi and bacteria resistant to heavy metals (silver, lead, zinc, manganese) isolated from mining waste of Guanajuato, Gto. Among the fungi we have isolated are strains belonging to the genera *Penicillium, Aspergillus, Paecilomyces, Cordyceps, Trichoderma* and *Fusarium*. Likewise, among the isolated bacteria are strains of the genera *Streptomyces, Bacillus, Brevibacterium, Microvirga* and *Comamonas.* These genera coincide with reports of microorganisms producing cellulases, among which are strains of fungi of the genera *Trichoderma, Aspergillus, Penicillium, Fusarium, Paecilomyces,* as well as strains of bacteria of the genera *Bacillus, Streptomyces* and *Cellulomonas* [1]. In this way, the general objective of this work was to analyze the cellulolytic activity present in fungi and bacteria from sites contaminated with heavy metals, which are microorganisms that have been able to survive in extreme conditions can present new enzymatic characteristics that are exploited biotechnologically. As specific objectives we have: 1.- Reactivate strains of fungi and bacteria isolated from sites contaminated with manganese, lead, zinc and silver. 2.- Detection of the cellulase activity in minimal solid medium with carboxymethylcellulose by the use of Congo red. 3.- Detection of the activity of cellulases in minimal liquid medium with carboxymethylcellulose by measurement of reducing sugars by the dinitrosalicylic acid (DNS) method. In this poster, the results obtained will be presented regarding the cellulolytic activity of the microorganisms analyzed.

Palabras clave: celulasas, metales pesados, rojo congo, ácido dinitrosalicílico.

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"Stabilization of Cytochrome P450 from Bacillus megaterium, through changes in positions C774A and C1000A in its Reductase domain"

Manuel Alejandro Arévalo Salina1, Felipe Olvera Rodriguez 2, Rodrigo Arreola Barroso 2, *Leticia Olvera Rodriguez2 and Gloria Saab Rincón2

Escuela de Ciencias UABJO1, Departamento de Ingeniería Celular y Biocátalisis, Instituto de Biotenología Universidad Nacional Autónoma de México2. Av. Universidad 2001 Col. Chamilpa 62210 Cuernavaca, Morelos, México. lolvera@ibt.unam.mx (777) 329-16-05.

Cytochromes P-450 are a family of hemoproteins that catalyze mainly NADPH-dependent monooxygenation reactions using molecular oxygen. One of the most significant characteristics is their low specificity, which allows them to metabolize a great number of compounds through including hydroxylation of aromatic and aliphatic compounds . This large variety of reactions makes cytochrome P450 an important tool for biocatalytic applications, such as the synthesis of pharmaceutical products or metabolites. A drawback for many of the members of this family of proteins is that they are anchored to the membrane and therefore have a limited solubility as well as a low stability; in addition the regeneration of the active site requires the interaction with specific reductases with a low electron transfer efficiency. In our laboratory we work with the cytochrome P450 from Bacillus megaterium (BM3), which has the advantages of being cytoplasmic, soluble and catalytically self-sufficient, since the catalytic and reductase domains are part of the same polypeptide chain, making the electron transport from the reductase domain to the heme domain more efficient. A detailed structural information is desirable to tailor the specificity of this protein, however there is no crystal structure of the entire protein and any attempt to crystallize it has been unfruitful. Efforts to increase the stability of this enzyme have focused mainly on the isolated heme domain, however, previous studies have indicated that the stability of this P450 is limited by its reductase domain, which exhibits a significantly lower stability compared to the heme domain. We were able to increase the stability of the reductase domain of P450 Bacillus megaterium previously by modifying six residues based on consensus (Mut M) (Saab-Rincon, G., et all., Chembiochem 2018); however, this mutant did not crystallize either. In order to increase the stability of this domain even further, we added two more mutations to MutM, which have been previously reported in the CytP450 (C773 and C999) that gave greater stability to the isolated reductase domain and that allowed its crystallization (Michael G. Joyce et all, 2012 FEBS Journal). In this work, the changes C773A and C999A (MutMC773A-C999A) were introduced in the complete protein stability of the protein was evaluated by thermal unfolding monitored by Circular Dichroism (CD). The results show that the P450 variant MutMC774A-C1000A shows a partial reversible folding after thermal denaturation. A comparison of the thermal unfolding behavior of this mutant and the parental mutant M is presented. Crystallizations assays are in their way.

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Purification and characterization of the extracellular laccase of Didymosphaeria sp. (syn.= Paraconiothyrium brasiliense sensu lato)

Marina Arredondo-Santoyo [1], Julio Cesar Villagómez-Castro[2], Ma. Soledad Vázquez-Garcidueñas[3] y Gerardo Vázquez-Marrufo[1*]

[1]Centro Multidisciplinario de Estudios en Biotecnología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Michoacana de San Nicolás de Hidalgo. [2] Div. de Ciencias Naturales y Exactas, Departamento de Biología, Universidad de Guanajuato. [3]Div. de Estudios de Posgrado, Facultad de Ciencias Médicas y Biológicas "Dr. Ignacio Chávez", Universidad Michoacana de San Nicolás de Hidalgo.

Extracellular fungal laccases are enzymes of biotechnological relevance. An extracellular laccase of the CMU-196 strain from *Didymosphaeria* sp. (syn. = *Paraconiothyrium brasiliense* sensu lato) induced with Cu^{+2} was purified to homogeneity in a three step protocol through chromatographic resins; the anionic exchange columns DEAE-Sepharose and MonoQ HR 5/5 were used followed by a molecular exclusion chromatography through a Superdex column. The molecular mass of the purified enzyme estimated by SDS- PAGE was 98 kDa. The biochemical characterization of the purified laccase reveals that the optimum pH is 4.5 using citrate buffer 0.1 M and that at 60 ºC, the enzyme shows the highest activity of 2251 (±16) U/L. The thermal stability of the enzyme was evaluated after 0.5 h□48 h incubation in citrate buffer at pH 4.5 and 60 ºC, showing that it is stable after 3h with a maximum activity of 76,887 (± 493) U/L. Thermal stability was also evaluated by pre-incubating the enzyme for 1 h in a temperature range of 4ºC□70ºC, showing that the enzyme loses activity as the temperature increases; laccase maintains 60% and 58% of the maximum activity at 20ºC and 30ºC, respectively. The ions Mg^{+2},Co^{+2}, Cu^{+2} induced enzyme activity at 1 mM, 5 mM and 10 mM, respectively; in the presence of Fe+2, activity decreased to 2.46% (±0.56) of relative activity. Inhibitors showed a decrease in enzymatic activity with respect to EDTA and EGTA at 1mM, with relative activity values of 38.27% (± 2.49) and 52.47% (± 3.5), respectively. In the presence of ABTS, the Km was 35.28 µM and a Vmax of 4943 mmol min^{-1} mg^{-1}; when the substrate is DMP, the Km was 14.11 µM and the Vmax of 787.2 mmol min^{-1} mg^{-1}, demonstrating that the enzyme has the ability to oxidize both substrates.

Key words: *Didymosphaeria* sp., laccase, purification, biochemical characterization.

Identification and characterization of the biotechnological potential of a wild strain of Didymosphaeria sp. (syn.= Paraconiothyrium brasiliense sensu lato)

Marina Arredondo-Santoyo[1], Ma. Soledad Vázquez-Garcidueñas[3] and Gerardo Vázquez-Marrufo[1*]
[1]Centro Multidisciplinario de Estudios en Biotecnología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Michoacana de San Nicolás de Hidalgo. [2]Div. de Estudios de Posgrado, Facultad de Ciencias Médicas y Biológicas "Dr. Ignacio Chávez", Universidad Michoacana de San Nicolás de Hidalgo.

The isolation and characterization of fungal strains from poorly described taxa allows undercover attributes of their basic biology useful for biotechnology. Here, a wild fungal strain (CMU-196) from *Didymosphaeria* genus was analyzed. CMU-196 metabolized 57 out of 95 substrates of the Biolog FF microplates. Efficient assimilation of dextrins and glycogen indicates that CMU-196 is a good producer of amylolytic enzymes. It showed a remarkably assimilation of a-D-lactose, substrate described as inducer of cellulolytic activity but poorly assimilated by several fungi. Metabolically active mycelium of the strain decolorized broth supplemented with direct blue 71, Chicago sky blue and remazol brilliant blue R dyes. The former two dyes were also well removed from broth by mycelium inactivated by autoclaving.
Both mycelia had low efficiency for removing fuchsin acid from broth and for decolorizing wastewater from the paper industry. CMU-196 strain showed extracellular laccase activity when potato dextrose broth was supplemented with Cu^{+2} , reaching a maximum activity of 46.8 (60.33) U L^{-1}. Studied strain antagonized phytopathogenic *Colletotrichum* spp. fungi and *Phytophthora* spp. oomycetes in vitro, but is less effective towards *Fusarium* spp. Fungi.
CMU-196 antagonism includes overgrowing the mycelia of phytopathogens and growth inhibition, probably by hydrosoluble extracellular metabolites. The biotechnological potential of strain CMU-196 here described warrants further studies to have a more detailed knowledge of the mechanisms associated with its metabolic versatility, capacity for environmental detoxification, extracellular laccase production, and antagonism against phytopathogens.

Keywords: *Didymosphaeria* sp., laccase, metabolism, bioremediation, antagonism

Characterization of the protein extract of the *Bromelia karatas* fruit and its antimicrobial activity against *Candida albicans*, *Salmonella typhimurium* and *Listeria monocytogenes*

Elva Avalos-Flores1, Denis Magaña-Ortíz[1], Elizabeth Ortiz-Vázquez [1*]
[1]División de Estudios de Posgrado e Investigación, Instituto Tecnológico de Mérida, Tecnológico Nacional de México, Av. Tecnológico km. 4.5 S/N, C.P. 97118, Mérida, Yucatán, México.
*Dirección: Av. Tecnológico km. 4.5 S/N, C.P. 97118, Mérida, Yucatán, México; Teléfono: 999 127 7178; e-mail: eortiz@itmerida.mx

Due to the increasing number of microorganisms resistant to antibiotics, the use of natural products as a source of new bioactive compounds has been considered as an alternative. Plants have compounds with biological activity and in certain species exhibit antimicrobial activity in their protein extracts. In the present work we propose the use of a soluble protein extract (SE) obtained from the fruit of the bromeliaceae *Bromelia karatas*. This species is commonly known in México as chichipo, chiyol, piñuela, aguama, timbiriche and cazuela. This plant is distributed in the Center and South of the country, where it has been used traditionally as fresh or prepared food for antiparasitic treatments. In this work, the SE of *B. karatas* was evaluated against *Candida albicans* ATCC 10231, *Salmonella typhimurium* ATCC 13311 and *Listeria monocytogenes* ATCC 15313. *B. karatas* fruit SE has showed a protein concentration of 9,612 µg/µL containing proteins of approximate sizes between 17 and 25 kDa, according to SDS-PAGE profile. In the other hand, it we established that SE does not present antimicrobial activity against *C. albicans* ATCC 10231; in contrast the extract showed antibacterial properties against pathogenic strains such as *S. typhimurium* ATCC 13311 and *Listeria monocytogenes* ATCC 15313. For *L. monocytogenes* the minimum inhibitory concentration (MIC) was 1700 µg/mL of protein present in SE. At this concentration, also bactericidal activity was observed. At the present time the value of MIC for *S. typhimurium* is still being determined with values above 1400 µg/mL. It was also possible to fractionate the SE using ion exchange chromatography, obtaining proteins of approximately 10, 12 and 20 kDa, which later will help us to identify the possible mechanisms which proteins give the antimicrobial activity. Based on the results obtained here, it can be suggested that SE does not present a fungistatic or fungicidal activity, but it has bactericidal activity against Gram-positive species.

Redox metabolites production of *Pseudomonas aeruginosa* NEJ01R using biodiesel process wastewaters

[a]Francisco Javier, Bacame-Valenzuela; [a]Jovita, Valdez-Paz; [a]Jesús Pérez-Garcia; [a,b]Yolanda, Reyes-Vidal.
[a]Centro de Investigación y Desarrollo Tecnológico en Electroquímica, S.C., Parque Tecnológico Querétaro s/n, Sanfandila, Pedro Escobedo, Querétaro. C.P. 76703; [b]CONACYT-Centro de Investigación y Desarrollo Tecnológico en Electroquímica. Parque Tecnológico Querétaro s/n Sanfandila, Pedro Escobedo, Querétaro, México. C.P. 76703.
fbacame@cideteq.mx; mreyes@cideteq.mx

The current problem related to the energy crisis due to the depletion of fossil fuels has driven research into alternatives for the replacement of fossil fuels (Anitha *et al.,* 2016). One of these options are the microbial fuel cells, a promising technology for the treatment of wastewater with electrical energy recovery. This technology uses microorganisms as biocatalysts to convert organic pollutants into electricity. Electrogenic microorganisms can grow on the surface of the anode using a biofilm, inducing the generation of electricity. However, some bacteria cannot transfer the electrons directly to the anode, so they produce redox mediators in solution, such bacterium *Pseudomonas aeruginosa.* This microorganism produces molecules of the phenazine group, such as pyocyanin (Chang *et al.,* 2014). The objective of this work was to use crude glycerol, a wastewater of biodiesel production as a substrate for pyocyanin production of *Pseudomonas aeruginosa* NEJ01R. Culture media were used with 1, 2, 3, 4 and 5% crude glycerol (CG) and pure glycerol (PG), sterilized in autoclave and inoculated with the study strain, incubated at 30 °C for 48 h, at 76 rpm, Luria Bertani (LB) medium was used as a positive control. The determination of pyocyanin was quantified after a liquid-liquid extraction and acidified with HCl, according to the method described by El-fouly *et al.* (2015). Glycerol consumption was determined by HPLC. It was demonstrated that *Pseudomonas aeruginosa* NEJ01R can use CG and PG as a carbon source, because glycerol is metabolized to dihydroxyacetone phosphate, metabolite that is an intermediate of glycolysis to later enter the Krebs Cycle for energy generation and other metabolites necessary for cell (Valkirea *et al.,* 2016). The concentration of biomass was higher in the treatment with 1% CG and PG, being respectively 8 and 33% for CG and PG of the biomass obtained with LB, with a glycerol consumption of 7 and 3% for CG and PG. The concentrations of piocyanine were also higher in the treatment with 1% of CG with 40% more than that produced with LB and only 30% of what was produced with LB was obtained when using PG. The results show that CG can be used as a substrate to produce redox metabolites of *Pseudomonas aeruginosa* NEJ01R.

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Fungi molecular identification of Tar Spot Complex on corn.

Rosibel Bahena Oregòn1, Yanet Romero Ramirez1, Miguel Angel Rodriguez Barrera 1, Daysi Návez González 1.
Facultad de Ciencias Químico Biológicas1, Universidad Autónoma de Guerrero1.
Lázaro Cárdenas Av. Ciudad Universitaria Sur. Chilpancingo, Guerrero, P. C. 39105,
Phone7471350008, biologia_rosi@hotmail.com.

The first report of the Tar Spot Complex was registered in Mexico in 1904 (Maublanc, 1904), it has caused economic losses up to 100% of the corn production in the country. The first symptoms of the disease appear with the presence of *Phyllachora maydis*, it has not been reported as a pathogen in other plant species. The second fungus involved is *Monographella maydis*, which forms a light brown elliptical halo surrounding every injury, this fungus causes the leaves and the completion the most significant damage (Hock et al., 1995), recently it was reported a *Curvuralia lunata* associated to the disease (Ríos et al., 2017). When the environmental conditions are favorable the fungi act in synergy destroying the crops in a lapse of 8 to 14 days. (Hock et al., 1995, Pereyda et al., 2009).
The objective is to identify the phytopathogens associated with the tar spot.
The samples were obtained by locality, from the estate Guerrero, municipality Chilpancingo. Were made fungi isolations involved in the disease from vegetable material with typical signs of the fungus disease, were placing, in culture medium, PDA, they were incubated in an environmental temperature with photoperiods of 18 h and were purified through tip hypha technique. The extraction of DNA was carried out by the method of Hoffman and Winston (1987). The identification was carried by the PCR technique, through the amplification of the gene 18s, with the oligonucleotides ITS1 (TCCTCCGCTTATTGATATGC) and ITS4 (CTTGGTCATTTAGAGGAAGTAA). They were compared to the sequences reported in the NCBI using the program BLAST.
Monographella maydis and *Fusarium solani* were identified as associated agents to the CMA in the samples present state of Guerrero.

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Overexpression of Dehydrin DHN1 and Rubisco Activase in Maize Plants Transformed by the Intragenic Method.

Raymundo Belmont Valadez[1], Verónica Garrocho Villegas[1], Lilia A. Bernal Gracida[1], Víctor Arturo González Hernández[2] and Estela Sánchez de Jiménez[1].

[1] Departamento de Bioquímica, Facultad de Química, UNAM, Ciudad de México, C.P.04510.
[2]Programa de Recursos Genéticos y Productividad, Colegio de Postgraduados. Km. 36.5 Carretera México-Texcoco, Montecillo, Texcoco, Estado de México, C. P. 56230
e-mail: estelas@unam.mx

Modification of target genes that might confer agronomic advantage to crops is a growing subject. Since drought is one of the anticipated abiotic stress factors as a result of global warming, because it would limit yield by reducing plant growth and biomass accumulation, it is the necessary to find genes that help the plants to tolerate this stress. On this regard a gene that has been reported to accumulate in maize plants under water deficit is dehydrin DHN1. On the other hand, Rubisco activase (RCA) is a gene that has been related to photosynthesis improvement and a higher grain yield. Therefore, DHN1 and RCA are target genes to improve maize plants by the intragenic method. This method, avoid the use of transgenes as well as selection genes, in order to maintain the genome free of transgenes and allows the genetic improvement of crops. This work focused into obtaining maize plants overexpressing RCA and DHN1 genes after the corresponding introduction of the intragenic constructions into embryogenic callus (E) by biolistic. We bombarded E calli with the intragenic constructions designed to overexpress RCA and DHN1, and thereafter we regenerate maize plants from this calli and transferred to greenhouse culture. We evaluated the incorporation of each construction by direct PCR and we also analyzed protein expression by Western blot. Results demonstrated that the intragenic constructions were integrated into the plants genome and that RCA was overexpressed constitutively, while the DHN1 construction was overexpressed solely by drought induction. Further analysis on drought tolerance and photosynthesis capacity were performed. Finally, we consider that the intragenic method is a promising technology to improve crops. although it still needs further development.

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Development of a recombinant protein GroEL of *B. canis*, for use as a diagnostic antigen for canine brucelosis.

Nancy Belem Beltrán Maldonado[1*], Rigoberto Hernández Castro[2], Erika Margarita Carrillo Casas[2], Alejandro Benítez Guzmán[1], Beatriz Arellano Reynoso[1]
[1]Faculty of Veterinary Medicine and Zootechnics.
[2]Hospital General Dr. Manuel Gea Gonzalez.
P.C. 04510, 56225896 ext. 14, arerey@yahoo.com.

Canine brucellosis is a zoonotic disease widely distributed worldwide, caused by *Brucella canis*[1]. In dogs it causes abortions, orchitis, epididymitis and iscospondylitis[2]
The diagnosis of canine brucellosis is made by direct methods such as: bacteriological culture and PCR (polymerase chain reaction) and indirect methods: tube agglutination test (TAT), agar double immunodiffusion (AGID)[3], counter-immunoelectrophoresis (CIEF),immunochromatographic assay (ICA), indirect immunofluorescence (IF), indirect enzymelinked immunosorbent assay (iELISA)[4.5] and rapid plate agglutination with 2ß-Mercaptoethanol (RSAT with 2ß-ME), teh further detects IgG and IgM antibodies[3] and is considered as a screening test, it shows levels of sensitivity of 70.5% and specificity of 83.3%[4].
The antigen used in the RSAT test is obtained from the *B. canis* reference strain (RM6 / 66 M),however, antigen production is carried out through a rigorous process in level III biosafety laboratories starting from bacterial cultures,and requires trained personnel for their interpretation. Additionally, in our country they are not produced commercially For this reason it is necessary to develop new biotechnological strategies to evaluate other antigens as potential candidates for the diagnosis of canine brucellosis.[6]
Therefore, a previous study performed in our laboratory evaluated an indirect ELISA test, using the immunodominant protein Hsp62, GroEL, obtained through Rapid Liquid Protein Chromatography (FPLC) with experimentally infected animals sera.[7]
The objective of this work is the development of a recombinant GroEL protein of *B. canis*, for the diagnosis of canine brucellosis.
The *groEL* sequence of *B. canis* was amplified to be cloned by enzymatic digestion into the vector pQE60 and expressed in *E. coli* M15 containing the plasmid pREP4 ; from this design we will obtain the recombinant clone containing pQE60-groel.
The protein will be tested by Western Blot and subsequently purified by FPLC (Fast Protein Liquid Chromatography) and it will be tested as the capture antigen in an iELISA, with serum from experimentally infected dogs.

Acknowledgements
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Removal of the Synthetic Dye Remazol Brilliant Blue R by Bioadsortion on Biomass from Aspergillus spp.

Irma Bernal Lugo,[1,*] Elisa Valenzuela Soto,[2] carolina Pedroza López,[2] Itzamna Baqueiro Peña,[2] Victor Contreras Jacquez,[2] Ali Asaff Torres.[2]
[1]Depto de Bioquímica, Facultad de Química UNAM. [2]Ciencia de los Alimentos, CIAD.
*irmofel@unam.mx

Introduction

Several industries (food, textiles, plastics, floriculture, pharmaceutical and others) generate large quantities of effluents contaminated with synthetic dyes. These represent a risk to human health due to their carcinogenic and mutagenic properties. They are also a risk for ecosystems and bodies of water because they reduce photosynthesis by limiting the penetration of light and are toxic to flora and fauna. At present, different methods have been developed for the removal of synthetic dyes from industrial effluents. Such as flocculation, filtration membranes, precipitation, chemical and enzymatic oxidation and adsorption. The latter process has proven to be highly efficient in the treatment of industrial effluents due to its high potential in the removal of organic matter and its low cost of implementation. The most widely used adsorbent is activated carbon; however, its high cost is a disadvantage when it is used on a large scale. Therefore, the use of biomaterials as adsorbents for the treatment of wastewater constitutes an alternative to the use of activated carbon.

Material and Methods

Four thermophilic strains named A45, B88, B38, B45 from the collection of the CIAD Biotechnology Laboratory were used. With these strains, experiments were carried out in submerged system for the bioadsorption of ramazol brilliant blue synthetic dye (RBBR). The effect of parameters such as the initial concentration of the dye, pH, bioadsorbent loading, contact time and temperature were evaluated.

Results and Discussion

Due to the morphological characteristics of the fungi in culture, the four strains belong to the genus Aspergillus. In order toknow if the removal of the dye from the solution was due to the phenomenon of bioadsorption and non by enzymatic oxidation, the activity lignin oxidases were quantified in the supernatant, but their activity was not detected. Therefore, we concluded that the removal of the dye from the solution was mainly due to the bioadsorption phenomenon. This was pH dependent and the system was saturated at a concentration of 500 mg/L of dye. The contact time between the bioadsorbent and the dye decreased as the loading of bioadsorbent was increased. The adsorption capacity of the strains was in the following order A88> A45 >> B38. The growth of strain B49 was not inhibited in the presence of the dye; but the strain did not exert any effect on the dye.

CONCLUSION

For the results shown the biomass of these strains could be used as adsorbent material in the removal of dyes from wastewater.

Neuronal localization assays of Rapamycin-loaded PLGA nanoparticles for potential treatment of Spinocerebellar Ataxia type 7

Fabiola Vianet Borbolla-Jiménez1, María Luisa Del Prado-Audelo2, Gerardo Leyva-Gómez2, Jonathan J Magaña1.
Instituto Nacional de Rehabilitación "Luis Guillermo Ibarra Ibarra". Adress: Calz México-Xochimilco 289, Arenal Tepepan, 14389 Ciudad de México, CDMX. Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Ciudad de México, 04510, México. Phone: 01 55 5999 1000 Ext. 14708. E-mail: maganasm@hotmail.com

Spinocerebellar Ataxia Type 7 (SCA7) is a neurodegenerative disease characterized by progressive cerebellar ataxia and retinal degeneration. This disorder has an autosomal dominant inheritance and is caused by a dynamic mutation that consists in an abnormal expansion of the CAG repeats in the coding tract of the *ATXN7* gene that encodes for the Ataxin-7 protein, which forms cellular aggregates within neuronal nuclei causing cell death. Currently, treatment for SCA7 is limited to symptomatic intervention, and there is no therapeutic approach to prevent or reverse the disease progression. Some mechanisms can be directed towards inhibition of the formation or enhancing the degradation of the protein aggregates, for delay or arrest the disease progression. However, successful treatment has been limited to date because of the intrinsic properties of therapeutic agents (poor water solubility, low bioavailability, poor pharmacokinetic properties), and difficulty in crossing physiological barriers, including the blood–brain barrier (BBB).

Therefore, in this study we present preliminary results of biodegradable nanoparticles (NPs) that could be used as nanocarriers of small molecules intended to improve its brain activity and also to test the hypothesis that protein aggregates may be eliminated by diverse mechanisms. For this purpose, poly-lactic-co-glycolic acid nanoparticles stabilized with poly(vinyl alcohol) with rapamycin encapsulated were produced and functionalized. The average NPs size was 243.13+3.06nm, polydispersity index of 0.06+0.02, and zeta potential of -17+0.17mV, showing an adequate stability and size. Then, the NPs were sterilized using UV and gamma radiation, proving effective activity against antimicrobial testing, nonetheless UV radiation appears to be a better choice, because it offers less damage to the molecular structure of the components. In addition, the NPs exhibited a high degree of compatibility in cell metabolic activity studies by MTT assays in glial and neuronal cells (MIO-M1 and SH-SY5Y, respectively). Hence, this study revealed that these NPs do not have toxic effects and we showed a high degree of the cellular internalization through of fluorescence analysis. These results suggest the high potential for the use of nanotechnology for the treatment of neurodegenerative pathologies such as polyglutamine diseases.

Bacillus thuringiensis as: growth factor and bioinsecticide helped by a synergistic protein

Martha Cecilia Bravo Rivas[1], *José Eleazar Barboza Corona*[1, 2], *Luz Edith Casados Vázquez*[1, 2]

[1]Master´s Program in Biosciences, Division Life Sciences, University of Guanajuato, Irapuato-Salamanca Campus. Irapuato, Guanajuato, 36500, Mexico
[2] Food department, Division life sciences, University of Guanajuato, Irapuato-Salamanca Campus. Irapuato, Guanajuato, 36500, Mexico.
cecy.bravoo@hotmail.com

Over time, the crops have been affected by abiotic and biotic factors, which together cause low yield and losses in total production. Solutions have been proposed and one of them is the use of growth factors to increase crop yields. There are reports about some bacteriocins acting as growth factor, thurincin 17 between them. It is remarkable to say that thurincin H is the same as thurincin 17 and in our lab we count with genetic cluster to produce it. On the other hand, to reduce losses caused by insect pests, bioinsecticides (Cry proteins) are used as biological control. However, cases of resistance to these bioinsecticides have already been detected. In an attempt to overcome this obstacle arose the use of synergistic agents such as chitinases (ChiA74) that facilitate the accessibility of cry proteins and therefore increase their activity. The objective of this work is to generate a *Bacillus thuringiensis* strain that acts as: growth factor and bioinsecticide helped by a synergistic protein. We transformed the constructions pcyt/ChiA74 and pThurH in *Bacillus thuringiensis* subsp. *kurstaki* HD1. After the transformation of both constructions it was observed that the thurincin H is synthesized after 12 hours and ChiA74 from 9 hours. Thus, a *Bacillus thuringiensis* subsp. *kurstaki* HD1 strain that synthesizes the cristal Cry1Ac, the chitinase ChiA74 and the bacteriocin thurincin H was obtained.

Keywords: crop, crystal, chitinase, bacteriocin, bioinsecticide, growth factor.

Development of monitoring oxygen and carbon dioxide transfer rate (OTR and CTR) device and cotton closure simulation during *Pichia pastoris* shake flask cultures

Daniel Cabrera Santos (danielcabsantos@gmail.com); Mauricio A. Trujillo Roldán (C.P. 04510; Tel. 56229192; maurotru@gmail.com).
Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México.

The methylotrophic yeast *Pichia pastoris* has been established as one of the most popular platforms for recombinant protein production due its advantages against other prokaryotic systems (i.e. very efficient secretion system, capability for postraslational modifications and innocuity for human health). Although there is a plethora of surveys dealing with the effect of operational variables over stoichiometric parameters of *P. pastoris* cultures, there is little information about the effect of oxygen and carbon transfer rate. In this topic, the obtained results reveal the lack of clear trends, suggesting that the effect of oxygen is a protein or strain dependent phenomena that could be monitoring by means of respirometric parameters as oxygen transfer rate (OTR), carbon dioxide transfer rate (CTR) and respiratory quotient (RQ). In this work we develop a device for monitoring the OTR, CTR and RQ of *P. pastoris* X-33 strain shake flask cultures. Then, to test our device, we measured and simulated the coefficient diffusion of cotton closures during *P. pastoris* cultures monitoring OTR, CTR, RQ and DOT and compare with conventional cotton closed shake flasks cultures in terms of DOT evolution, optical density and specific growth rate (μ). The reliability of our system was tested with glucose as carbon source, the results agree well with that obtained with RAMOS system.

Our system comprises a valves system which supplies air satisfying the measured coefficient diffusional values, probes for oxygen and carbon dioxide concentration in the gas phase (located in the shake flask headspace) and a device for dissolved oxygen tension (DOT) measure in liquid phase as well as equations for calculation of OTR, CTR and RQ from concentration data. Then, we measure the diffusion coefficient of three different closures: conventional cotton closures (mass=5.9 g, vol=62.8318 cm^3, ρ=0.0939 g/cm^3), 0.00853±0.00145 mol O_2/h; microfiber filter with pore size of 0.2 μm, 0.00646±0.00049 mol O_2/h and PTFE filter, 0.0163±0.00049 mol O_2/h. The measured diffusion coefficient of cotton closure, which corresponds to airflow of 0.018 L/min, was chosen for simulation with the valve system. The DOT behavior was very similar in cotton closure simulation and closed with real cotton closure cultures, starting oxygen limitation conditions at the 8 hours and extending for about 60 hours. Importantly, statistical analysis indicated that it doesn't exist differences in terms of specific growth rate: 0.2214 y 0.2427 h-1, for real cotton closed cultures and simulated cotton cultures, respectively.

We found that the OTR range values: 40-60 mmol/L h and CTR range values: 40-50 mmol/L h, indicated an oxygen limitation process observed as a typical increase-plateau-decrease shape plot described in other works. The RQ range value of 0.80-0.85 agrees with theorical values of glycerol (0.85). Finally, the reliability of our system was tested with glucose as carbon source, we observed the same metabolic response observed during *P. stipitis* cultures with RAMOS system.

Extraction and chemical and functional characterization of squalene, starch and protein of *Amaranthus* species.

Oscar de Jesús Calva Cruz[1], Alberto Barrera Pacheco[2], Ana Paulina Barba de la Rosa[2].

[1] Instituto potosino de investigación científica y tecnológica a.c. División de biología molecular. Camino a la Presa San José 2055. Col. Lomas 4ta sección CP. 78216. San Luis Potosí S.L.P. Tel: (444) 834 20 00. Ext 2083.Correo: apbarba@ipicyt.edu.mx

Introduction: Amaranth (*Amaranthus spp.*) is a native grain of Mexico, whose importance has resurged in the past 20 years because of its agricultural features, since it is a fast-growing cultivar with tolerance to drought conditions. Owing to their significant starch, protein, and lipid content, amaranth seeds can be considered promising raw materials to produce flour, starch and protein concentrates. High-carbohydrate flours have been obtained from amaranth seeds by an enzymatic process, which might find application as a drymilk extender and sweetener. Moreover, the amaranth seed has remarkably amounts of squalene; which has shown important nutritional and nutraceutical properties. Amaranth grain has an excellent concentration of proteins (13%–19%) with high digestibility (90%). Due to the high protein concentration, amaranth could be a source of bioactive peptides with biological activities such as anti-hypertensive, cancer preventive, anti-thrombotic, among others. Nowadays, the methodologies described for the extraction of starch, squalene and proteins have several limitations, such as the recovery of one or two of them. In addition, reagents used are toxic, generate pollution or require expensive infrastructure, limiting the maximum use of amaranth seed. **Objective**: The objective of the present work was to obtain the three main compounds in amaranth seeds: an oily fraction rich in squalene, the starch fraction and protein, an evaluate its potential as a food ingredient.

Methodology and results: A methodology were developed to recover amaranth seed lipids, starch and proteins in sequence. Initially, the amaranth seed was cleaned and ground to remove the seed coat. The conditions of pressure, temperature and volume of CO_2 necessary to extract with high performance the lipids contained in the sample were determined. The lipid extract was analyzed and characterized by gas chromatography - mass spectrometry (GC-MS): the yield was 75 to 80% of squalene, in addition to the recovery of palmitic, stearic, linoleic and linolenic acid. The defatted seed was soaked and milled with NaOH at low concentrations and after a few hours it was sieved to separate and recover the insoluble fiber. The sieved sample was subjected to centrifugation for the recovery of the starch in the sediment. The rheological and granule size properties of the starch were characterized using scanning electron microscopy. The pH of the recovered supernatant was decreased to pH 5 by the addition of hydrochloric acid to precipitate the proteins dissolved in the medium, and 80% of the total protein was recovered. Polyacrylamide gel electrophoresis (SDS-PAGE) was performed and the proteins observed were characterized by Liquid chromatography – mass spectrometry (LG-MS/MS). These assays have been tested at the pilot plant level and the results were reproducible at large volumes.

Conclusion: The results show the recovery of squalene, starch and proteins from the same sample; as well as a minimum amount of losses. Considering that the method uses non-toxic reagents, easily accessible, low cost, and compatible with applications in the food industry, the explotation potential of amaranth in this, and other areas of commercial interest increases significantly.

Influence of *SNF1* gene on glycolytic flux addressed to aerobic fermentation (Crabtree effect) of *Saccharomyces cerevisiae*

Andres Carrillo-Garmendia, Miguel David Duffo-Hurtado and Luis Alberto Madrigal-Perez
Laboratorio de Biotecnología Microbiana, Instituto Tecnológico Superior de Ciudad Hidalgo, Ciudad Hidalgo, Michoacán, 61100, México. Email: lmadrigal@itsch.edu.mx

The alcoholic fermentation is a biotechnological process, which has been employed for more than one hundred years. Despite the ample researches carry out about this, there are specific aspects are still not fully understand, an example of this is the Crabtree effect. The Crabtree effect is a metabolic phenomenon that occurs when the concentration of fermentable sugars in the medium is higher than 0.015% (w/v) and is characterized by the increase of glycolytic flux, an augment in the production of metabolites of fermentation and respiration repression. In spite of the wide quantity of information on the Crabtree effect is still no clear the molecular basis by which originates. It has been proposed that sucrose non-fermenting protein-1 (Snf1p) plays a key role in the unleashing of the Crabtree effect. Since in high concentrations of glucose, Snf1p is found dephosphorylated; while in low concentrations of glucose the protein Snf1p is phosphorylated and participate in the de-repression of genes repressed by glucose. However, it has been suggested that both Snf1p phosphorylated and dephosphorylated have an essential participation in the metabolic transition of fermentative metabolism to the respiratory metabolism. This study aimed to evaluate the influence of the deletion of *SNF1* gene in the glycolytic flux addressed to the Crabtree effect of *S. cerevisiae*. For this purpose, we use the *Saccharomyces* cerevisiae genetic background BY4742 and its mutant in the gene *SNF1* (*snf1Δ*). Here, we provide evidence that *snf1Δ* mutants grown in YPD medium supplemented with 10% glucose did not present any effect upon glycolytic flux. In this regard, NADH/NAD+ ratio, extracellular acidification rate (ECAR) and transcription levels of genes *HXK2* and *PFK1* did not show a significative difference between strains *snf1Δ* and wild-type. Surprisingly, the strain with the deletion in the gen *SNF1* exhibit an increased oxygen consumption in comparison with wild-type. The results obtained in this study suggest by first time that even when Snf1p is found dephosphorylated in high concentrations of glucose could be necessary for carrying out the repression of the respiration.

Morphological study of dedifferentiation process on *Rosmarinus officinalis* leaf explants by digital image analysis

Yessica Casales-Tlatilpa, Araceli Urquiza-López, Cresencio Bazaldua, Pablo Emilio Vanegas-Espinoza, Pérez-Mendoza Martha Berenice, Alma Angélica Del Villar-Martínez*. Instituto Politécnico Nacional, Centro de Desarrollo de Productos Bióticos. Yautepec, Morelos, México. C.P. 62731. Tel. (735) 394 20 20 ext. 82526 *adelvillarm@ipn.mx

Rosemary (*Rosmarinus officinalis* L.) is an aromatic plant that produces compounds of interest for pharmacy, cosmetic and food industry. Digital images analysis allows quantifying morphological changes that occur during explants dedifferentiation process. The aim of this work was to evaluate morphometric changes on leaf explants during cellular dedifferentiation process. Cellular dedifferentiation of leaf rosemary explants was induced on MS medium supplemented with 2, 4-dichlorophenoxyacetic acid (1.5 mg/L), and 6-benzylaminopurine (2.0 mg/L). Digital images were acquired (three per week), with a scanner, for thirty days. Digital image analysis was done using ImageJ® software in order to quantify changes on explants (area and perimeter), during cellular dedifferentiation process. This analysis allowed identifying three growth stages during the studied process, the first from days 0 to 4, with an increment of area (1.44 mm^2/d) and perimeter (0.86 mm/d), related with explants adaptation to the culture media. The second growth stage, from days 7 to 23, was related with cells dedifferentiation by the induction process, with a lower increment on both registered parameters, area (1.27 mm^2/d) and perimeter of 0.53 mm/d). The third stage was related with an active cell division of dedifferentiated cells, with an increase of 2.8 mm^2/d and 1.08 mm/d for area and perimeter respectively, for days 25 to 30. Digital image analysis allowed identifying growth stages, during rosemary leaf explants dedifferentiation process, based on quantitative changes on area and perimeter.

Characterization chromatography of the avocado seed

[1]Dolores Castañeda-Antonio.[2]Fabiola Vázquez Salinas, [2]Aldo Avelino Maldonado [2] Deysi Santamaría-Juárez [1]Ricardo Munguía-Pérez. [2] M. Elena Ramos-Cassellis, [1]Jesus Muñoz-Rojas, [1]Elizabeth Y. Morales García and Yuriria Páez Santoyos
[1] Instituto de Ciencias, CICM Benemérita Universidad Autónoma de Puebla, Ed.11 IC Laboratorio de ecología Molecular Microbiana C.U., Fracc. Jardines de San Manuel Puebla, Puebla, México. C.P. 72590
[2] Facultad de Ingeniería Química, Benemérita Universidad Autónoma de Puebla
E- mail dcastaneda.antonio@gmail.com

RESUMEN
Topic area: Biotechnology
Introduction: In the avocado Hass wa much in demand, Mexico is the first exporter of avocado, the consumption of this one is increasing as a consequence of its progressive incorporation to the alimentary habits of new consumers of all the scopes when being a product of high nutritional quality, It has very important functional properties, however, it is still necessary to take advantage of its qualities.
Objective: To determine the functional compounds present in the avocado seed through gas chromatography coupled to masses.
Methodology: Various extractions of the avocado seed were performed for the extraction of the compounds, determination of solid weight of the seed, concentration of extracts by rotavapor and analysis in a 7890 Agilent Technologies chromatograph and identification of compounds present through the NIST Library 08
Results: 13-Octadecenal (Z) was the one with the highest presence in ethanol with 53.72%, 2-Dodecylcyclobutanone followed by 6-Octadecenoic acid with 6.53%, as well as in chloroform 13-octadecanal with 49.80% in chloroform 2-Dodecylcyclobutanone with 16.27%, in hexane continuous being the most abundant followed by Estragole with 21.83%, these compounds have a high functionality for human metabolism as well as the treatment of dyslipidemias.
Conclusion: Due to the presence of the various compounds, we can propose a pharmaceutical use for antioxidant, anti-inflammatory and cytotoxic properties and phenolic composition present in the avocado seed. In the avocado seed these remarkable properties have been found as well as some other pharmacological properties due to the high amount of fatty acids (Werman and Neman, 1986). In pre-Columbian times this seed has been used against conditions such as muscle pain, eliminating the presence of parasites and as an antifungal agent..

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Citric acid effect in fungal tolerance to chromium stress

Oscar-Guadalupe Castillo-Martínez[1]; Fernando Martínez-Armenta[1]; César Álvarez-Mejía[2] and Varinia López-Ramírez[1]

[1]Instituto Tecnológico Superior de Irapuato. Coordinación de Ingeniería Bioquímica. Carretera Irapuato - Silao km 12.5, C.P. 36821. 2Instituto Tecnológico Superior de Abasolo. Blvd. Cuitzeo de los Naranjos #401, Col. Cuitzeo de los Naranjos, C.P 36976. valopez@itesi.edu.mx. Tel. 462 6067900 Ext. 141

Lichens are symbiotic associations between a fungus or mycobiont with a yeast and an algae or a cyanobacterium. It has been shown that mycobionts have the ability to tolerate heavy metals such as hexavalent chromium. Citric acid is a reported organic acid with antioxidant and chelating capacity in plants under heavy metals stress.

In this project, we evaluated the effect of citric acid on chromium VI tolerance by two fungal isolates, M008 and *Flavoparmelia sp.* like, with the aim of determined if citric acid could increase their ability to heavy metal stress tolerance and also analized the secondary metabolites produced during this process. The fungal isolates were inoculated in Sabouraud Dextrose Agar (SDA) with chromium (1-6 mM) and with chromium at three different concentrations of citric acid (80, 100, 125 mg/L), radial growth was measured every 24 hours and tolerance index were calculated through growing difference between chromium, chromium plus citric acid and controls. The identification of secondary metabolites was performed by Thin Layer Chromatography (TLC) trough qualitative analysis of Retention Factors (RF's). The presence of citric acid at concentrations of 80, 100 and 125 mg/L in the medium did not inhibit the growth of fungal isolates. We observed a greater tolerance to chromium in isolate M008, which could increase its tolerance up to 6 mM. We observed that *Flavoparmelia sp*. increased its tolerance at 1 mM and 3 mM. The highest tolerance index for isolate M008 was obtained in the presence of citric acid (125 mg/L) and for *Flavoparmelia sp*. in 1 mM chromium was in the presence of citric acid (80 and 100 mg/L) being different in the presence of 3 mM chromium where the tolerance index was affected in the presence of citric acid at the three different concentrations, the pattern of bands in the TLC results in the presence of citric acid shows great similarity with SDA banding without chromium and without citric acid. These results show there were no difference in secondary metabolites synthesis, most of the metabolites found according to the RF for the eluting solution were xanthones. Our results show that citric acid has a positive effect on chromium tolerance of the two fungal isolates. This study suggests that citric acid could be used in bioremediation techniques such as biostimulation.

Neutralizing capacity of a hyperimmune sera from *Crotalus durissus terrificus* snake venom conjugates

Irene Maria Castillo Perez[1]; Alejandro Olvera Rodríguez[1]; Irving Giovanni Archundia Jimenez[1]; Melisa Benard Valle[1]; Edgar Enrique Neri Castro[1]; Hilda Vazquez Lopez[1]; Felipe Olvera Rodriguez[1]; Alejandro Alagon Cano[1].
[1]Instituto de Biotecnologia UNAM, Departamento de Medicina Molecular y Bioprocesos. Av. Universidad #2001, Col. Chamipa. C.P. 62210 Cuernavaca, Morelos. Tels: +52 (777) 329 16 49, (55) 56 22 76 49, +52 (777) 311 49 00 ext 294. E-mail: irenecas@ibt.unam.mx

Key words: Neutralizing capacity, *C. durissus terrificus*, antivenom, crotamine, conjugates.

In America rattlesnakes of the genus Crotalus are considered of medical importance due to their wide distribution and the high levels of morbidity and mortality that they cause. Envenomations by some Crotalus rattlesnakes are related to the presence of crotoxin and crotamine, which cause neurotoxic and myotoxic symptoms.
Crotamine is a cationic polypeptide composed of 42 aminoacids with a molecular weight of 4.8 kDa. Despite its lower lethality compared with crotoxin, crotamine has an important myotoxic and cytotoxic effect. In mice, crotamine is able to induce paralysis of the hind limbs due to its interaction with some Kv channels.
One of the major limitations in the successful development of crotalid antivenoms is the poor immunogenicity of crotamine due to its low molecular weight. Also, the variable concentration of crotamine in the venom of rattlesnakes used to produce this antivenoms, affect the generation of antibodies against crotamine. In consequence, most of the crotalid antivenoms produced in recent years failed to recognize and neutralize crotamine. This problem can contribute to the need of higher antivenom doses in patients.
A good strategy to increase the immunogenicity of a polypeptide is to produce conjugates using cross-linking reagents. In this study, we produced and characterized conjugates with the proteins in the venom of *C. d. terrificus,* using different cross-linking reagents. We produced, in rabbits, experimental hyperimmune sera with these conjugates and analyzed their neutralizing capacity and the recognition of the venom components of different rattlesnakes.

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Cofactor Specificity Engineering of a Water-Forming NADH Oxidase from *Giardia lamblia* as Universal Regenerating System for Redox Reactions

Adriana Castillo Villanueva, Paola Ramírez Fonseca, Omar Salinas Pérez,
Sara Teresa Méndez, Jesús Oria Hernández*
Laboratorio de Bioquímica-Genética. Instituto Nacional de Pediatría. Secretaria de Salud.
Insurgentes Sur 3700-C, Insurgentes Cuicuilco; Coyoacán, Ciudad de México. *☎(5255) 1084 0900 Ext. 1442 *✉ jesus.oria.inp@gmail.com

Enzyme catalyzed redox reactions have recently gained increasing interest in biocatalysis. The NAD(P)H Oxidase family is a group of soluble oxidoreductases catalyzing the oxidation of NADH or NADPH by reducing molecular O_2 to H_2O, H_2O_2 or both. We have previously characterized the NADH oxidase from the parasite *Giardia lamblia* (GlNOX)1. This enzyme can use NADH or NADPH as electron donors and oxygen as the primary electron acceptor, rendering H_2O as end product without production of O_2.- or H_2O_2. Unlike other members of this family, which use selectively only NADH or NADPH, GlNOX can use both cofactors with slight preference of NADH over NADPH (NADH V_{max} 63.4 ± 4.1 µmol min^{-1} mg^{-1}, K_m = 17.7 ± 2.9 µM; NADPH V_{max} = 36.4 ± 1.8 µmol min^{-1} mg^{-1}, K_m = 87.4 ± 10.1 µM). The capacity of enzymes like GlNOX to oxidize both NADH and NADPH with side products no other than water has been highlighted for its potential as versatile and innocuous regeneration systems for redox reactions requiring NAD^+ or $NADP^+$ as cofactor. In this work, we attempt to decrease the selectivity of GlNOX for the cofactors in order to allow this enzyme to be used as a universal regenerating system for redox reactions. To this end, three single point mutants were constructed (D180A, G181R and S182H) which has been shown to be implicated in the selectivity of the cofactors in others members of this family. Mutants were successfully constructed and purified. Steady state kinetic measurements show that mutations alter the kinetic parameters of the enzyme, albeit at different extents. It is expected that by combining single mutations we can obtain an enzyme that uses both cofactors with similar efficiency.

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Composite coating of AgNP'S with antimicrobial activity

Berenice Castro Rodriguez, Francisco Javier Bacame Valenzuela[a], María Yolanda Reyes Vidala[b], Gabriel Trejo Córdova
[a]Centro de Investigación y Desarrollo Tecnológico en Electroquímica, Parque Tecnológico Querétaro s/n Sanfandila, Pedro Escobedo, Querétaro, México. C.P. 76703; [b]CONACYT-Centro de Investigación y Desarrollo Tecnológico en Electroquímica. Parque Tecnológico Querétaro s/n Sanfandila, Pedro Escobedo, Querétaro, México. C.P. 76703.
gtrejo@cideteq.mx, mreyes@cideteq.mx

Metal nanoparticles such as gold and silver have a wide variety of applications in health, environment, agriculture and diagnostics. They are defined as solid materials with at least two dimensions between 1 and 100 nm. Silver nanoparticles (AgNP's) have been used as an antimicrobial agent against Gram-positive and Gram-negative bacteria. It is reported that ions and silver products have a toxic effect for more than 16 species of bacteria (Ke et al., 2018, Acharya et al., 2018). One of the applications of the AgNP's is as metallic alloy coatings for the design of functional surfaces. Therefore, in this work the antimicrobial capacity of an AgNP's coating was evaluated on a Cu-Zn matrix, obtaining a composition (Zn-Cu / AgNP's) at a concentration of AgNP's of 0%, 0.24% and 0.28% against *Staphylococcus aureus* ATCC65389 and *Escherichia coli* ATCC10536. 24-hour cultures of both strains were used in Luria Bertani medium at 30° C and 150 rpm. After the incubation period, the composites were added for a time of 1, 5 and 10 minutes. Taking aliquots of the culture medium in each period of time, serial dilutions were made with each aliquot by selecting 107 to perform the plate count method. For the composition without AgNP's, a percentage inhibition for *Staphylococcus aureus* was 3.5% at a contact time of 1 and 5 minutes, increasing to 9% at a time of 10 minutes. For the composition with 0.24% of AgNP's, it achieved an inhibition percentage of 1.7% at minute 1, increasing to 29% at minute 5 and reaching 50% for 10 minutes. For the composition with 0.28% of AgNP's the inhibition was 2% at minute 1, 53% at minute 5 and 95% at minute 10. With the cultures of *Escherichia coli* the inhibition was up to 23% with the composition without AgNP's. However, with 0.24% of AgNP's an inhibition of 17% was achieved for 1 minute, 35% for 5 minutes and 38% for 10 minutes. When 0.28% of AgNP's was used, 100% growth was inhibited. The results obtained show that the use of Zn-Cu/AgNP's composites can decrease the growth of pathogenic microorganisms, being useful for the design of functional surfaces. The inhibitory effect was analyzed by concentration levels of AgNP's.

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Secondary metabolites from *Metarhizium with effect on bacteria*

Karla Yadira Cervantes-Quintero, Juan Carlos Torres-Guzmán, Israel Enrique Padilla-Guerrero and Gloria Angélica González-Hernández.

Departamento de Biología, División de Ciencias Naturales y Exactas, Universidad de Guanajuato. Noria Alta s/n, zip code 36050, Phone number: 7320006, Ext.8160 e-mail gonzang@ugto.mx

Antimicrobial resistance (AMR), is an of the most significant actual problem around the world in the health sector. O'Neill on 2014 report that resistance to antibiotics will increase towards 2050 resulting in millions of deaths per year.

Antibiotics are drugs widely used in different medical treatments and procedures in humans, and its use in the meat production industries has been widely popularized.

The increase in AMR problems lies in the inappropriate use of these drugs and the lack of new therapeutic compounds for antibacterial treatment, therefore has increased the frequency of appearance of resistant bacteria (Alanis, 2005; Banin *et al*., 2018).

Plants and microorganisms are attractive sources for the discovery of new therapeutic compounds. At present, many secondary metabolites produced by entomopathogenic fungi, such as *Metarhizium*, are still unknown (Spatafora and Bushley, 2015). *Metarhizium* spp. have different lifestyles: saprophyte, endophyte, insect pathogen, plant disease antagonist and plant growth promoter (Staats *et al*., 2014; Gibson *et al*., 2014; Sasan and Bidochka, 2012). This metabolic flexibility is an excellent opportunity to find new compounds with biotechnological potential.

In *Metarhizium* has been reported different secondary metabolites like helvolic acid (Lee *et al*., 2008), isocoumarin glycosides (Tian *et al*., 2016) and euvesperins A and B (Siina *et al*.,2016).

In this work, we searched for secondary metabolites in nine *Metarhizium* strains to find new metabolites that's help in the treatments against bacteria. *Metarhizium* strains were grown 120 hours in different culture media and collect the supernatants, and we prove it in two Gram-negative bacteria (*E. coli* and *Salmonella*) and one Gram-positive bacteria (*Bacillus subtillis*). The fungi secreted products and bacteria cultures were incubated for 24 hours in 96 well plate and measuring the decrease in population with Optical Density at 600 nm. Currently, we are in the process to identify the secreted secondary metabolites by HPLC-mass spectrometry.

Silencing and overexpression of the aquaporin gene *pvpip 2-4* affect nodulation in *Phaseolus vulgaris*

Mariana Esther Cesario Solis, Marco Adán Juárez Verdayes, Alfonso Leija Salas, Yolanda Ortega Ortega, Olivia Santana Estrada, Daniel Tapia Maruri, Carmen Quinto Hernández, Luis Cárdenas Torres. Instituto de Biotecnología/Dpto. Biol. Mol. de Plantas. Universidad Nacional Autónoma de México. Cuernavaca, Morelos, México. CP 62271. Tel. 777329 1666 Email: msolis@ibt.unam.mx

Aquaporins are widely spread in animal and plant cells. Plant aquaporins constitute a large and diverse family of water channel proteins that are essential for several physiological processes in living organisms. Numerous studies have linked plant aquaporins with the transport of other solutes such as NH4, sugars, among others, but the H2O2 transport have been recently related with other important physiological processes such as plant growth and development, and to biotic and abiotic stresses responses. Since the H2O2 have a key role in plant signaling as a second messenger, the mechanisms regulating its generation and distribution in plant cells have been considered a key regulatory point. Aquaporins could regulate the strength of the H2O2 signal, but also regulates the subcellular distribution. For instance, they have been localized in the plasma membrane, endoplasmic reticulum, vacuoles, plastids and, they can even localize in membrane compartments interacting with symbiotic organisms such as symbiosomes. It has been proposed that aquaporins can transport hydrogen peroxide, however, little is known about this process. Since mutualistic interactions require a regulated reactive oxygen species (ROS) production during the infection process, we anticipate that aquaporin could play a key role during this process. Polar growth requires the activity of NADPH oxidase which generated superoxide in the extracellular plasma membrane of the apical dome in tip growing cells such as pollen tubes and root hairs. However, this superoxide is dismutated to H2O2 and then transported by an unknown mechanism to the cytoplasmic side. We have considered that aquaporins could be involved in this mechanism during polar growth, but also during the mutualistic interaction since nodulation requires the control of tip polarity during the invagination of the plasma membrane, and cell wall for the infection thread formation, a tunnel like structure. This structure allows the colonization of the host plant by the rhizobium symbiont. Furthermore, aquaporins could play a role during the nodule development, which also requires the activity of NADPH oxidase, the ROS generating enzyme. However, little is known about the aquaporins family in common bean and their role in mutualist associations, such as the rhizobia-legume symbiosis. The objective of this study was to determine the role of *PvPIP2-4*, a gene encoding an aquaporin that could be involved in the H2O2 transport. By silencing and overexpression of the gene in *Phaseolus vulgaris*, we have evaluated the effect on the nodulation process. We have found that transcript for *PvPIP2-4*, accumulated abundantly in roots which have been inoculated for 3, 5 and 7 dpi with *Rhizobium tropici* CIAT899; however, at 14, 21 and 30 dpi, the level of transcript decreased considerably. The results regarding, subcellular localization, nodulation and nitrogen fixation will be presented and discussed.

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Isolation of bacteriophages for the treatment of *Pseudomonas aeruginosa* infections

María I. Chávez Béjar, Jesús Hernández Romano, Carolina Lagunes Delgado, Esmeralda Badillo Ocampo, Karla de la Rosa Morales
Universidad Politécnica del Estado de Morelos, Av. Cuauhnáhuac 566, Col. Lomas del Texcal, Jiutepec, Mor. CP. 62550. mchavez@upemor.edu.mx.

The discovery of antibiotics increased the life expectancy of human beings, since many diseases considered deadly at the beginning of the 20th century, nowadays are treated successfully. However, It is very common that several bacterial species have resistance to several of the antibiotics for clinical use, so it is difficult and expensive to treat the infections caused by these microorganisms. An example of this is *Pseudomonas aeruginosa*, an opportunistic pathogen, which is one of the causative agents of nocosomial diseases in immunocompromised patients.

It has been shown that the use of bacteriophages is an excellent alternative for the treatment of bacterial diseases since they infect the bacteria in a specific manner. For this reason, in our working gruoup, several bacteriophages were isolated from wastewater in order to fight infections caused by *P. aeruginosa*. The viruses were characterized by their lysis rates, host range, and their stability in different solutions that allow their application and long-term storage. According with the results obtained in this study, the bacteriophages isolated in this study fulfill several ideal characteristics to be used in phagetherapy.

Detection of Intestinal Bacteria in Drinking Water and Residuals.

Lorena Chávez González, Simón Guzmán León, José Luis Santillán Torres, Jorge Ramírez Salcedo.

Unidad de Microarreglos de DNA, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México. Circuito exterior, Ciudad Universitaria, Coyoacán, A.P. 70-242, México, D.F. CP. 04360. Tel: 56225753. http://microarrays.ifc.unam.mx. Correo electrónico: lchavez@ifc.unam.mx

The supply of drinking water in Mexico is a service that is responsibility of the municipalities, in each of the states of the country; within these there are operating organizations that are directly responsible for supplying water in sufficient quantity and with a quality that complies with the Official Mexican Standard, the basic purification system includes procedures that remove turbidity, bacteriological and chemical contamination mainly. Most of the supply in our country comes from groundwater (aquifers) and in lower percentage surface water (rivers, dams and lakes). A few years ago, groundwater was preferred because of its high quality, currently it already has leaking problems, and contamination by fecal coliforms has already been found. Total and fecal coliforms are widely used worldwide as indicators of potability because they are easy to detect and quantify; but they are no longer so reliable, it has been shown that the absence of traditional indicators of potability, do not ensure the absence of pathogens, the water may be contaminated by another group of microorganisms that respond differently to disinfection. Although the supply of drinking water has increased, gastrointestinal diseases are the main health problem in our country, with children being the most vulnerable group. Therefore it is necessary to implement more reliable tests that allow us to evaluate the quality of water for human consumption. Here we describe a methodology that has been developed in the DNA Microarray Unit, which allows us to specifically identify the pathogen that is contaminating. Using the membrane filtration technique, large volumes of water are concentrated, then the membrane dissolves to release the bacteria trapped in the filter. The cells are broken by heat to release the DNA and an aliquot is taken to make a PCR reaction in multiple format that incorporates a fluorescent molecule. The amplicons are hybridized in a DNA microarray that has probes printed in the form of digital numbers to identify 19 enterobacteria, with the possibility of designing a microarray containing the microorganisms of interest. It is a low cost test and the results are obtained from 24-48 hours. Additionally the slide is printed in multiple format (12 arrangements), therefore 12 different samples can be tested at the same time under the same conditions, with equipment that can be transported to the place where it is required without major problem.

Molecular characterization of *Pycnoporus* spp

Lizbeth Coronel Pastor[1], Ma de Lourdes Acosta-Urdapilleta[2], Elba Villegas[1]
[1]Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos,
[2]Centro de Investigación en Biología, Universidad Autónoma del Estado de Morelos,
Av.Universidad N. 1001, cl. Chamilpa, Cuernavaca, Morelos elbav@uaem.mx

Introduction. Fungi degrade lignin and cellulose which leads to decompose the wood. Because of these capacity they can be classified into three types, the ones that cause brown, soft or white rot. *Pycnoporus* belongs to white rot fungi that produce lacasses which has several applications in the biotechnological area such as: bioindicator of the durability of wood, in the paper industry, bioremediation, in addition, they produce metabolites with antioxidant, anti-carcinogenic, antiviral, anti-fungal, anti-inflammatory and antimicrobial properties. Laccases are also involved in pigmentation, formation of the fruiting body and fungi pathogenicity.
Specifically *P. coccineus* has been reported to produce phenoxazinone synthase,an enzyme that participates in the biosynthesis of the the fenoxazinone ring which is the basic molecule to produce actinomicine D. The aim of this work was to identify by molecular biology 50 strains of *Pycnoporus* by molecular biolgy and distinguish *P. coccineus* t from *P. sanguineus, P. cinnabarinus and P. pucniceus* identification four different genes: ITS, β-tubulin, laccases and phenoxazinone synthase [1]. Materials and methods. The strains were seeded in HIT agar medium for maintenance and other media to favor the induction of the ITS, β-tubulin, laccase and phenoxazinone synthase genes based on Lesage-Meessen, 2011 [2].
DNA extraction was performed follwing Lomascolo et al., 2002 [3]. PCR amplification was done with pre-designed primers of the ITS, ß-tubulin and laccase genes, while primers for the fenoxazinone synthase gene were designed based on the sequences obtained from NCBI [http: //www.ncbi.nlm .nih.gov]. The PCR products were sent for sequencing to the Synthesis and Sequencing Unit, IBTUNAM. Finally, phylogenetic analyzes were obtained from the individual genes using the program MEGA7.0.26.
Results. Among 50 *Pycnoporus* strains 13 strains of *Pycnoporus cinnabarinus*, 13 strains of *P. sanguineus*, as well as 4 strains *P. coccineus* were identified however 10 more strains they remain unidentified.
Conclusion. Phylogenetic trees obtained may help to undertand the relationships among them, it will be interesting to explore the relatin ships among other *Pyconporus* strains from around the our cuntry México.

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Genetic characterization and selection of rhizobacteria from *Agave americana* L for use asbiofertilizers.

Néstor Hugo Cruz Pérez*, Reiner Rincón Rosales, Víctor M Ruíz Valdiviezo
Instituto Tecnológico de Tuxtla Gutiérrez, Carr. Panamericana km. 1080, CP 29050, Tuxtla Gutiérrez, Chiapas.Tel: 9611424113.
nhcp_10@hotmail.com

ABSTRACT.
The objective of this research was to study and analyse the diversity of bacteria associated with the *Agave americana* L., a plant of agro-industrial importance in Chiapas, México, since this plant produces a traditional drink called "Comitéco" and as a source of sucrose type fructans. For this purpose, rhizobia strains with high potential for their use as biofertilizer were selected. The strains collected were characterized phenotypic and genotypically. The taxonomic identification and classification of isolates was carried out by means of a phylogenetic study using the 16s rRNA gene, genomic fingerprinting and nifH plasmid study. The symbiotic potential of the selected rhizobacteria was evaluated through in-vitro and exvitro bio-fertilization tests.

Key words: *Agave americana*, rhizobacteria, biofertilization, molecular markers.

INTRODUCCIÓN.
Agave americana is a species that belongs to the Agavaceas family, has been adapted and grows in the border region and the highlands of Chiapas, where it is used to obtain a drink called "Comitéco". The study of the bacteria associated with plants of agricultural importance, has increased recently, considering the potential of these microorganisms for their use as biofertilizers. One of the main reasons for the search for microbial species as biofertilizers, is due to ability of some bacteria to fixing N2, a fundamental element for plant growth, Martínez,*et al*., (2005). This study aims to study the genetic diversity of rhizobacteria associated with *A. americana* and select strains with high potential for use as biofertilizers, using microscopic techniques and characterization genotypic.

MATERIALS AND METHODS.
A total of 34 samples were collected in experimental plots located in the Comitan, Chiapas. 17 samples were of rhizosphere soil and 17 of the roots. Soil samples were collected for physicochemical study. The disinfection of roots was according to, Bashan et al (1993). The soil was dilute until a concentration of 106. The samples were inoculated in the NFB nitrogen-free medium and the isolated strains were cultured in Rojo Congo medium. The strains were verified through a Gram stain and microscopically analyzed the cellular structure.
The DNA of strains was extracted using ZR fungal/Bacterial Microprep™ kit of ZYMO Research to later carry out the PCR of the gen 16s rDNA. The pcr products were sequenced and compared in the GenBank to obtain their taxonomic assignation. Representative strains were selected to evaluate the symbiotic potential through in vitro and ex vitro biofertilization tests, using a completely random design.

RESULTS.
The highest percentage of the strains showed rapid growth in the culture medium. Form of Gram-negative bacilli and different morphology of the colonies formed in Rojo Congo medium. From strains that amplificacon for the gen 16s rDNA were obtained sequences of the strains representing the different groups (table 1), and were compared with database of NCBI. Phylogenetic trees were built for analysis and genetic identification.

Género	Especie	No. de cepa
Achromobacter	*A. marplatensis*	4a.
	A. sp	8, 172
	A. xylosoxidans	35
Agrobacterium	*A. tumefaciens*	5, 6, 19, 26, 27b, 62, 143
Acinetobacter	*A. sp*	13a, 109
	A. calcoaceticus	40, 67, 145
	A. junii	183
Novosphingobium	*N. sp*	14a, 14b
Pseudomona	*P. putida*	17b, 21a, 54, 106
	P. gingerii	53
	P. sp	138
	P. mosselii	140
	P. plecoglossicida	210
Rhizobium	*Rh sp/ Sh sp*	34a.
Enterobacter	*E. asburiae*	98
Klebsiella	*K. oxitosa*	99
Comamonas	*C. testosteroni*	97
Stenotraphomonas	*S. maltophilia*	31b, 157
	S. sp	116

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Expresión, purificación y caracterización fisicoquímica y estructural del dominio catalítico de la MMP-16, potencial blanco terapéutico contra Aterosclerosis y Cáncer.

Armando Cruz-Rangel[(1);] Paulina Santiago Rubio[(1)] ; Juan Pablo Pardo-Vázquez[(3)] ; Gabriel Gutiérrez-Magdaleno[(2)]; Marco Antonio Loza Mejía.[(4)]; Carolina Suárez[(4)] & Juan Pablo Reyes-Grajeda *[(1)].

Adscripción: (1) Instituto Nacional de Medicina Genómica. Periférico Sur 4809, Arenal Tepepan, 14610 Ciudad de México, CDMX, acruz@inmegen.gob.mx, jreyes@inmegen.gob.mx; (2) Universidad Autónoma Metropolitana U. Xochimilco; (3) Dpto. Bioquímica Facultad de Medicina UNAM.(4) Facultad de Ciencias Químicas, Universidad La Salle.

RESUMEN

La metaloproteasa de membrana 16 (MMP16/MT3-MMP) es una endopeptidasa dependiente de Zn^{2+} que ha cobrado relevancia en diversos escenarios patológicos como el cáncer y la aterosclerosis. En dichos contextos, su importancia radica en la activación mediante proteólisis de la proMMP2/progelatinasa, así como por su actividad degradadora de múltiples componentes de la matriz extracelular. Su sobrexpresión se ha relacionado con el crecimiento y capacidad invasiva de diversos tipos de carcinomas como son: próstata, mama, melanomas y carcinoma hepatocelular, haciendo de ella un potencial blanco para el diseño de fármacos. Sin embargo, el alto grado de homología entre las diferentes MMPs hace difícil el tener inhibidores selectivos para cada una de estas.

El objetivo de este trabajo fue realizar la caracterización bioquímica-estructural del dominio catalítico de la MMP16 humana, para sentar las bases en el diseño racional de fármacos con alta especificidad para esta.

Resultados: Se desarrolló un protocolo para la sobrexpresión y purificación del cdMMP16 humano producido en un sistema heterólogo procarionte (E. *coli*). Mediante DLS en solución, se encontró un arreglo monomérico de 90% (23 KDa) con 10% de fracción dimérica (43 KDa) del cdMMP16 humano purificado. La caracterización por fluorescencia intrínseca muestra cierto grado de termoresistencia de la enzima en presencia de sus cofactores (Ca^{2+} y Zn^{2+}); así como, por el batimastat y autoproteólisis a altas concentraciones. Las determinaciones cinéticas de la enzima con concentraciones crecientes de sustrato fluorescente FS-6 mostraron una Km= 10.50 µM y una Vmax de 173 s^{-1}. La IC50 de inhibición del batimastat para el cdMMP16 fue en un rango de 250-500 nM lo cual evidencía la potencia del inhibidor aunque no sea selectivo. Conclusiones: 1. Se obtuvo el dominio catalítico de la MMP16 humana con altos rendimientos. 2. La proteína presenta estructura secundaria definida, similar a la reportada para otras MMPs. 3.Los espectros de fluorescencia intrínseca muestran termo-resistencia de la enzima en presencia de sus cofactores Ca^{2+} y Zn^{2+}, así como por el batimastat. 4. Los metales Ca^{2+} y Zn^{2+} podrían promover actividad autoproteolítica, mientras que en ausencia de dichos metales, la enzima evidencia intermediaros oligoméricos de alto peso molecular; lo cual sugiere un posible mecanismo de regulación. 5. Los ensayos de actividad e inhibición para la proteína sientan las bases para estudios de interacción proteína-ligando de compuestos selectivos deducidos *in silico* mediante acoplamientos moleculares (docking).

Hydrocarbon degrading activity of a bacterial consortium isolated from sediments of the Gulf of Mexico.

Diego Humberto Cuervo Amaya[1], María del Refugio Trejo Hernández[2], Daniel Morales Guzmán[2], Fidel Alejandro Sánchez Flores[1], Grisel Alejandra Escobar Zepeda[1], Liliana Pardo López [1].

[1]Instituto de Biotecnología – Universidad Nacional Autónoma de México, [2]Centro de Investigación en Biotecnología – Universidad Autónoma del Estado de Morelos.

Av. Universidad #2001, Col. Chamilpa C.P. 62210, Cuernavaca, Morelos. +52 777 3291600 ext. 38137. liliana@ibt.unam.mx.

The bacterial degradation of hydrocarbons in contaminated areas is the most important natural mechanism that contributes to removal of oil. The presence of hydrocarbons in the environment diminishes the microbial diversity, selecting the microorganisms that are capable of resist and assimilate the high concentrations and variety of carbon chains. Bacteria or bacterial consortiums that have a hydrocarbon degrading activity are desirable to evaluate if an ecosystem under contaminated by oil can respond positively to pollution. For this purpose, a sediment sample (308 m deep), from hydrocarbon-contaminated sites on the southwestern coast of the Gulf of Mexico, was grown in the laboratory. To promote the selection of degrading bacteria, a mixture of hydrocarbons was used as carbon source (medium crude oil with kerosene as a dispersant) in a mineral broth enriched with phosphorus and nitrogen. Using amplicons of the 16S gene it was identified that the presence of hydrocarbons favored the enrichment of the *Gammaproteobacteria* class, especially of the genera *Alcanivorax*, *Halomonas* and *Idiomarina*, accumulating up to 97 % of the relative abundance; these genera are related to the degradation of hydrocarbons in contaminated areas. The bacterial population went from 918 genera in the environmental sample (Shannon-Weaver index: 6,982) to 55 genera after eight weeks of cultivation (Shannon-Weaver index: 1,891); in this time, up to 54 % of the hydrocarbons supplied were degraded, together with the environmental loss (34 %). In conclusion, bacterial genera from deep sediments of the Gulf of Mexico were identified, with the capacity to grow with hydrocarbons as the sole carbon source.

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Obtaining 3-hydroxyanthranilic acid from the liquid culture of *Pycnoporus cinnabarinus* for the synthesis of antibiotics

Elizabeth Cuevas-Reyes[1], Elba Cristina Villegas Villarreal [*1]
[1]Biotechnology Research Center, Av. Universidad No. 1001, Col Chamilpa, Cuernavaca, Morelos, Mexico. C.P. 62209.
elbav@uaem.mx*

Pycnoporus cinnabarinus, is an intense red fungus, which grows on dead wood in tropical and subtropical weathers. It has been reported to produce various metabolites among them 3-hydroxyanthranilic acid (3HAA) and cinnabaric acid (CA). 3HAA is the precursor of CA, which has antibiotic activity towards Gram positive and negative bacteria. CA structure is similar to actinomycin D because both compounds have a phenoxazine ring and can be synthesized from the same precursor when the precursor is modified with a pentapeptide. The biosynthesis of both compounds is mainly mediated by a laccase or in some cases by anther enzyme phenoxazine synthase. CA have numerous applications in different areas such as: food, cosmetics, bioremediation, pharmaceutical, among others and Actinomicine D was used first as an antibiotic but now its been used as anticancer drug. *P. cinnabarinus* has been cultivation in different lignocellulosic and defined media to obtain mainly laccases and CA. The question arose as to whether it is possible to optimize the culture medium changing glucose, yeast extract and $CuSO_4$ concentration in the media to improve the production of 3HAA. Lacasas and CA. In this study, the optimization of a defined media was done according to a 2^K factorial design method to increase 3HAA, laccase and CA production by *P. cinnabarinus*. Experiments changing concentration levels of glucose, yeast extract and CuSO4 concentration were performed using 25 different media by triplicates in 125 mL flasks up to 15 days for *P. cinnabarinus* cultivation at 28 C and 150 rpm. The yields of 3HAA and CA were determined by reverse phase HPLC and Laccase activity by spectrophotometry using 2,6-dimethoxyphenol (2,6-DMP) as a substrate. Culture filtrate antimicrobial activity was determined by agar diffusion plate on Müller Hinton agar against *Escherichia coli* and *Staphylococcus aureus*. Among all media assayed three of them stand up, the first one for 1,183.1 µg/mL 3HAA production, the second one for 3.4 µg/mL cinnabarinic acid yields and the last ne for the highest yields of laccase activity registered with up to 519,500 U/L. Antimicrbial activity was determined towards *E. coli* and *S. aureus* using all crude extracts obtained from the liquid culture media, where a bacteriostatic effect was observed in all of them. Laccase activity zymograms were obtained to determine which laccase is able to synthesize antibiotic compounds. Nevertheless it is necessary to continue to purified and sequence the laccases responsible for the synthesis of these structures with antibiotic activity. In addition, in a new future modified 3HAA precursor could serve for the synthesis of other new antibiotics.

Protective effect of acetonic extract of leaves of *Ficus* sp on oxidative damage in HaCaT cells

DeLaCruz
Brenda de la Cruz Concepción, Mónica Espinoza Rojo, Juan Miguel Mendoza Bello, Patricia Álvarez Fitz, Napoleón Navarro Tito, Ana Elvira Zacapala Gómez, Miguel Ángel Mendoza Catalán.
Autonomous University of Guerrero; Faculty of Biological Chemistry Sciences. CP 39000. Cel.: 7471008594, correo electrónico: brenddc@hotmail.com.

Introduction

Oxidative stress causes potential biological damage in macromolecules and a wide variety of physiological changes in cells[1]. Plants have constituted since ancient times an invaluable resource for the care and preservation of health. 15% of medicinal plants have been investigated to determine their biological activities and the responsible active compounds, which demonstrates the extensive medicinal potential they possess, one of these medicinal activities is the antioxidant2. Between the plants studied for their extensive antioxidant activity, related to the inhibition of ROS and the maintenance of the redox state, is the genus *Ficus*[3], however, Mexican species have not been analyzed despite their wide distribution in the country.

Objective

To evaluate the effect of the acetonic extract of *Ficus* sp leaves (AEF) on oxidative damage in HaCaT cells.

Methodology

The antioxidant activity of the extract was determined with the DPPH and ABTS technique. The effect on viability in HaCaT cells was evaluated by the MTT assay. H_2O_2 was used to induce oxidative damage (lipoperoxidation) and the 1-methyl-2-phenylindole method was standardized to quantify malonaldehyde levels. **Results and discussion**

The CI50 of the ABTS shows that the extract has antioxidant activity similar to the results reported in several species of *Ficus,* to which the antioxidant activity has been attributed to the high content of polyphenols and flavonoids[4]. AEF concentrations of 5, 10 and 20 µg/mL has no effect on the viability of HaCaT cells, while at concentrations higher than 40 µg/mL the extract significantly decreased cell viability. The concentration of 600 µM H_2O_2 produces high levels of MDA with respect to the control.

Conclusions

The AEF exhibited significant antioxidant activity. The AEF at concentrations of 5, 10 and 20 µg/mL does not affect the viability of HaCaT cells. The concentration of 600 µM of H_2O_2 induces oxidative damage in the cell line.

Key words

Ficus sp, MDA, reactive oxygen species (ROS), DPPH, ABTS.

Acknowledgments

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Prospecting and characterization of psychrophiles microorganisms plant growth promoters.

Irán Tapia Vázquez[1], Jorge Luis Folch Mallol[1], Ramón Alberto Batista García[2], Ricardo Sánchez Cruz[1]. [1] Centro de Investigación en Biotecnología, [2] Centro de Investigación en Dinámica Celular, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos CP. 62210. (777) 3 29 70 00 ext. 4550 iran.tapia01@gmail.com

Key words: P*sychrophiles, xinantecatl, plant growth promotion.*

Introduction. The current trends in agriculture, which focus on the reduction of chemical pesticides and inorganic fertilizers have prompted the search for alternatives that can sustain agricultural yield and environmental quality. An important means of achieving this is through the use of microbial inoculants that have a bearing on soil health and plant growth promotion (Selvakumar, *et al*. 2007).
Glaciers harbor a wide diversity of microorganisms, metabolically versatile, highly tolerant to several environmental stresses and potentially useful for biotechnological purposes (Balcazar, *et al*. 2015).
Nowadays, there is little information about the microorganisms that live in extreme Mexican enviroments and less still of those that could be good promoters of the vegetable growth in conditions of low temperature, being the yeasts the least studied group.
The characterization of plant growth promoting psychrophiles bacteria and yeasts would be an important contribution for the development of cold active bio fertilizers or phyto stimulants that can satisfy the agricultural needs of warm and cold cultures and are capable of counter the adverse effects that the low temperatures generate on the hot cultures.

Methodology. Three rhizosphere samples of native plants near to the crater area of the Xinantécatl volcano (P1, P2 and P3) and a sample of snowy soil (N) were taken to obtain microorganisms. For the isolation of the microorganisms, Sabouroud and PDA media were used free and supplemented with Kanamycin. The isolated strains were submitted to qualitative biochemical tests related to the processes of plant growth promotion: Indoleacetic acid, siderophores, cellulases, xylanases and proteases production and phosphates solubilization. All isolates were tested *in vitro* for germination and growth promotion of tomato seedlings (*Solanum lycopersicum*). The effect of each of the strains was also tested as possible biocontrol agents of phytopathogenic fungi. Work is currently under way on the determination of the identity of each of the strains.

Results: Twelve microorganisms of apparently different morphology were obtained. Currently, these bacteria and yeasts strains are identified by a key name referring to the collection area from they come: BP2.1, BP2.2, BP2.3 and BN (Bacteria); LP1.1, LP1.2, LP1.3, LP1.4, LP1.5, LP3.1, LP3.2, LP3.3. To each of them, qualitative biochemical tests were done to determine their plant growth promoter characteristics. Several promising strains were detected that showed significant positive effects on the germination time and growth promotion of the tomato seedlings compared to the control plants.
Likewise, they showed effects of up to 50% inhibition of the growth of four phytopathogens: *Rhizoctonia solani*, *Fusarium* sp, *Phytophthora capsici* and *Alternaria solani*.

Conclusions. With the obtained data, the strains LP1.1, LP1.5, BP2.1 and BN have been selected as promising plant growth promoters that are used for the microbial formulation that can be tested *in vivo* cultures.

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Microbial growth phases of *Bacillus thuringiensis* characterized by online permittivity and conductivity measurements.

Escalante-Sánchez, Abdi; Barrera-Cortés, Josefina; Ríos-Leal Elvira; Ponce-Noyola T.; Farrera-Rebollo Reynold

Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Departamento de Biotecnología y Bioingeniería, Av. Instituto Politécnico Nacional 2508, Gustavo A. Madero, San Pedro Zacatenco, 07360 Ciudad de México, CDMX
abdi.escalante@cinvestav.mx; jbarrera@cinvestav.mx ,

Abstract

The dielectric analysis of cell suspensions is a useful technique that allows the online estimation of biomass concentration based on measurements of permittivity and conductivity of the culture broth (Kiviharju et al., 2008). This technology has been applied in the bioinsecticide production process by *Bacillus thuringiensis* allowing the online detection of the microbial growth phases (Sarrafzadeh et al., 2005) and the implementation of strategies for the controlled addition of nutrients in fed-batch culture mode (Escalante-Sánchez et al., 2018). However, the in-depth analysis of biomass estimations and conductivity measurements may provide real-time insight of important metabolic events that could be used to develop a more sophisticated control strategy for process optimization. In this work, the growth metabolism of *Bacillus thuringiensis* was investigated by means of on-line permittivity and conductivity measurements. Several batch cultures with complex media formulations were performed under different conditions to assess the signal profiles. The estimation of the kinetic variables of the process (μ, $YF_{X/S}$, q_s, and q_x), allowed to observe the metabolic state of the microorganism up to the sporulation. The conductivity signal was useful in pH-controlled experiments for the detection of the accumulation and assimilation of organic acids during the exponential growth phase. A decline in the conductivity signal that correlated with the decline in specific growth rate typical of the transition phase between exponential growth and sporulation (R2>0.9 over all the batch experiments) suggests that conductivity variations in the culture media are indicative of the metabolic state of the microorganism.

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Structural features involved in the hemolytic activity of a pepper Defensin

Georgina Estrada, Cynthia Martinez-Liu, Lucila Sanchez Cach.
Centro de Investigacion Cientifica de Yucatan. Unidad de Bioquimica y Biologia Molecular de Plantas. Calle 43 No. 130. Colonia Chuburna de Hidalgo, CP 97200, Merida, Yucatan, Mexico. Tel. (999) 9428330.
correo electronico: georgina.estrada@cicy.mx

Plants are sessile organisms that are exposed to numerous biotic or abiotic stress factors. As innate immune response, defensins are antimicrobial peptides conserved among plants, invertebrates and vertebrates. Most plant defensins are acidic and antifungal peptides with 45 to 55 amino acid residues and eight cysteines that form disulfide bridges. Structurally, plant defensins belong to the alpha beta cysteine stabilized family (αβCS), as other cysteine rich peptides. The region near the C-terminus; formed by the loop between the beta sheet 2 and beta sheet 3, and known as the γ-core, is the motif that interact with phospholipids for peptide internalization through membranes. CADEF1, is an antifungal defensin from *Capsicum annuum.* It is defined as a pepper defensin, but its primary sequence is highly conserved in plants. By recombinant expression and a two step purification protocol we obtained a soluble form of the His tagged CADEF1. The mutantpeptide in the γ-core of CADEF1 (CADEF1_AAAA$^{35\text{-}38}$) was also expressed in bacteria and showed reduced hemolytic activity in comparison to the wild type CADEF1. Differences of dimer abundance were observed between the wild type and the mutant peptide. Results of the hemolytic assays indicate that the γ-core is involved in the hemolytic activity of CADEF1 and constitutethe first evidence of interaction with human membranes of this pepper defensin.

Restructuring of the Rhizospheric Microbiome of *Musa acuminata* (banana) Cultivated in Southeastern Mexico, upon Soil Inoculation with Microbial Consortia

Ali Asaff-Torres[1*], Elisa Fernández-Castillo[1,2#], Jorge Lara[1#], Armando Mendoza[1#], Enrique Avendaño[1], José María Gaxiola[3], and Jorge Verdín[2*]

[1]Innovak Global, Chihuahua, Chihuahua, Mexico; [2]CIATEJ-Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C., Zapopan, Jalisco, Mexico. [3]Rancho Sonora, Teapa, Tabasco, Mexico. [#]These authors contributed equally to this work.
aasaff@innovakglobal.com; jverdin@ciatej.mx

Musa acuminata is the fourth most important crop in the world after rice, wheat and corn. Because of its economic importance, *M. acuminata* is subject of intense scientific research to increase its performance, as well as to find new strategies to face its main pathogen, *Fusarium oxysporum*. Due to its influence on the development, growth and health of the plants, the study of the rhizospheric microbiome of *M. acuminata* is critical to stablish strategies that intelligently modulate the rhizospheric microbiota to increase the crop health and productivity. To that end, it is particularly relevant to understand how rhizosphere microbial populations are influenced by different abiotic and biotic factors such as soil structure, weather conditions, and the introduction of new microbial species into the rhizospheric community. The aim of this study is to characterize and model the dynamics of the rhizospheric microbiome of *M. acuminata* along its development utilizing a metataxonomic approach. The plants studied in this work are being grown in two farms in Southeastern Mexico (Tapachula, Chiapas, and Teapa, Tabasco), both established in different soils and exposed to different climate. Additionally, plants are being systematically amended with a consortium of fungi and bacteria (BioFit, INNOVAK, Mexico) or mycorrhizal fungi and bacteria (MycoRoot, INNOVAK, Mexico). Besides verifying the effects of soil and weather variability on the productivity and characteristics of the rhizospheric microbiome of the plant, we will determine the efficiency of incorporation of the consortium in the rhizosphere, as well as their effect on the architecture of the native rhizospheric microbiota along the development of the plant. The results of this study will be presented at the congress. This work is supported by Innovak Global, Chihuahua, Mexico.

Evaluation of toxic activity of reactivated *Bacillus thuringiensis var israelensis* from fermentation extracts, after more than three decades of storage and analysis of toxic persistance against *Aedes aegypti* larvae

Q.B.P. David Daniel Fernández Chapa, Dr. Luis Jesús Galan Wong, Dra. Graciela García Díaz, Dra. Katiushka Arévalo Niño, Dr. Hugo Alberto Luna Olvera.

Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, Instituto de Biotecnología. Ave. Pedro de Alba s/n cruz con Ave. Manuel L. Barragán s/n, Cd. Universitaria, C.P. 66455, s\n San Nicolás de los Garza, N.L daviddanielfdz@hotmail.com

Introduction: The toxins of *B. thuringiensis israelensis* have shown great potential in the control of harmful insects. Such is the case *Diptera* as the mosquito *Aedes aegypti*, who represent a serious threat to public health because of their hematophagous nature and vector capacity. H. Dulmage established diverse methodologies for mass production, product formulation, and power standardization. From these processes, several hundred fermentation extracts of cultures belonging to this microorganism, were donated by the United States Department of Agriculture in 1989 to our research group. Since then, these extracts have been kept in the Universidad Autónoma de Nuevo Leon (UANL) International Collection of Entomopathogenic Bacilli. Particularly important within this collection are 5 fermentation extracts, which we decided to utilize in this research to prove their ability to kill mosquitoes under laboratory conditions through their residual activity comparing and evaluating the toxicity of reactivated strains. **Objetive**: To determine the toxicity of the reactivated and stored Bti extracts for more than 3 decades against Aedes aegypti larvae. **Hypothesis**: The reactivated strains of HD Bti will have great toxicity against the Aedes aegypti mosquito compared to Bti stored strains for more than 3 decades will still have persistent biological activity. **Methodology**: The stored Bt strains were reactivated and the presence of crystals was observed by optical microscopy after the protein crystals were purified by the lactose-acetone co-precipitation method and the biological activity of stored and reactivated extracts against Aedes aegypti was determined according to the protocol of Official Mexican Standard NOM 032 2015. **Results**: Stored Bti fermentation extracts showed persistence of the toxic activity of Bti against *A. aegypti* after three decades of storage. All the extracts studied showed toxicity at the highest doses tested (0.1 ppm) and some of the extracts, from strains 3260 and 3501, killed larvae at doses as low as 0.02 ppm stored and reactivated had an LD50 of .001ppm and .07ppm respectively in comparison of the same stored extracts where their LD50 were 0.12ppm and 1.16ppm. **Conclusions:** Bti protein crystals can prolong their toxicity in shelf life or in field conditions under specific conditions. New crystals of reactivated Bt extracts are more effective than stored ones. Strain 3260 with LD50 of .002 shows great potential to develop as biopesticide.

Carotenoid production in cultures of *Phaffia rhodozyma* under magnesium limited conditions

[1]Iris Isabel Flores Manzanero, [1]Luis Bernardo Flores Cotera

[1]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional Unidad Zacatenco
Av. Instituto Politécnico Nacional 2508, Col. San Pedro Zacatenco, Delegación Gustavo A. Madero, Ciudad de México, México. C. P. 07360.
Tel: +52 (55) 57473800. iris.flores.manz@cinvestav.mx

Phaffia rhodozyma (sexual status, *Xanthophyllomyces dendrorhous*) is a sort of yeast that has the ability to synthesize several carotenoids, and in a greater abundance astaxanthin, which has shown to be a powerful antioxidant. The environmental and nutritional conditions regulate the synthesis of carotenoids in yeast, which protect the cell from the damage caused by reactive oxygen species. It has been demonstrated that the synthesis of astaxanthin takes place under oxidative conditions, among other conditions, when growth is restricted due to a metabolic impediment. Magnesium is a key element necessary for the function of a diversity of enzymes related to the synthesis of ATP, so the main objective of this work was to analyze the relationship of the deficiency of the element with the synthesis of carotenoids. The experiments were carried out with strain NRRL-Y-10922, using a defined medium whose sole magnesium source was $MgSO_4 \cdot 7H_2O$ (limiting conditions 0.07 mM, without limitation 0.6 mM) in a 3 L reactor. Biomass (dry weight), concentration of total carotenoids, proteins, sugars, ethanol and percentage of dissolved oxygen were analyzed. As expected, the magnesium deficiency promoted the production of carotenoids accompanied by a lower growth of the microorganism and a lower synthesis of proteins. A mechanism was proposed whereby magnesium deficiency is related to oxidative stress

A novel alcoholic functional beverage made from *Agave salmiana* and probiotic mixed culture

Alejandro García Cruz[1], Irving Rendón Mendoza[1], Miguel Angel López Rosario[1], Hugo Rosales Bravo[1,2], María Yolanda Mercedes Rodríguez Aza[3], Georgina Berenice Cueva Torres[3], Horacio Claudio Morales Torres[3], Victor Olalde Portugal[3.]

[1]Departamento de Ingeniería en Biotecnología, Universidad Politécnica Metropolitana de Puebla, Puebla, Pue. 72480. Correo: alejandrogarciacruz691@gmail.com.
[2] Departamento de Nutrición, Universidad del Valle de Atemajac Campus Tepic, Tepic, Nay., 63168. Correo: rbhugo@hotmail.com.
[3] Departamento de Biotecnología y Bioquímica, CINVESTAV Unidad Irapuato, Irapuato, Gto., 36824.

ABSTRACT

In this study the functional potential of a microbial consortium in fermentation of sterile and non-sterile *Agave salmiana* mead was evaluated. The microbial consortium was formed by a wild yeast isolated from a natural fermentation of Pulque and three probiotic *Lactobacillus* strains (*Lactobacillus acidophilus* ATCC 4356, *Lactobacillus paracasei* ATCC 25302, *Lactobacillus rhamnosus* ATCC 53103). The wild yeast was sensory selected in fermentation of sterile mead by a panel of untrained panelists, to which the alcohols profile produced were determined by HPLC method, while the probiotic strains were selected according to their clinical application. The functional potential of the microbial consortium was determined according the production of essential free amino acids by the HPLC method in fermentation of sterile and non-sterile *Agave salmiana* mead under aerobic and static conditions, at 30 ° C for 24, 48 and 72 h, using as controls sterile mead and artisanal Pulque. The data was analyzed using the analysis of variance test and comparison of means by the Tukey method at a level of 95% confidence, using the statistical package MINITAB version 17. The results indicate that the wild yeast produced 77.9% less methanol than the commercial Pulque ($P < 0.05$). The ethanol content was similar ($P < 0.05$), while for the production of higher alcohols: propanol, butanol, isoamyl alcohol, isobutanol and phenylethanol, the wild yeast stood out with respect to the controls ($P < 0.05$). For the essential amino acids profile, the consortium excelled in the production of Ile (3.19 ± 0.32 mg/l) in fermentation of non-sterile mead at 24 h of fermentation ($P < 0.05$), and in the production of Trp (42.76 ± 2.12 mg/l) in sterile mead at 72 h of fermentation ($P < 0.05$). Our results suggest the functional potential of the beverage produced by the consortium under controlled fermentation conditions, generating a product with probiotic and nutritional effect on the consumer.

Opuntia ficus-indica nanofibers as drug release system

Elsy J. García-Valderrama, Hector Manuel Leija Gutiérrez, Karina del Ángel Sánchez, Narsimha Mamidi, Marilena Antunes-Ricardo, Janet A. Gutiérrez
[1]Centro de Innovación en Diseño y Tecnología, Ave. Eugenio Garza Sada 2501 Sur, Col. Tecnológico C.P. 64849, Monterrey, N.L., México
[2]Centro de Biotecnología-FEMSA, Tecnológico de Monterrey, Avenida Eugenio Garza Sada 2501 Sur, 64849 Monterrey, NL, Mexico

Introduction*: Opuntia ficus-indica* (OFI) is a xerophyte plant which belongs to the dicotyledonous angiosperm *Cactaceae* family. The main phytochemicals within the OFI include carotenes, ascorbic acid, phytosterols, chlorophyll, betaxanthins, taurine and flavonoids, being isorhamnetin glycosides the most abundant flavonoids. Biological effects such as hypoglycemic, antioxidant, apoptotic, antibacterial, antifungal and anti-inflammatory activities have been attributed to the isorhamnetin glycosides. In the present study, crosslinked gelatin fibers loaded with isorhamnetin glycosides from OFI powder were successfully produced using the *Forcespinning* method which utilizes centrifugal force to spin fibers. **Materials and methods**: The *Forcespinning* was performed under different parameters to obtain high yield of isorhamnetin glycosides, fiber homogeneity, and small diameters. Likewise, in order to improve their water-resistant ability and thermomechanical performance for potential biomedical applications, the gelatin fibers were submitted to a crosslinking process using saturated glutaraldehyde (GTA) vapor at room temperature. The content of isorhamnetin glycosides in the fibers was analyzed by HPLC-PDA. The release rate of isorhamnetin glycosides from the crosslinked gelatin fibers was also assessed. **Results**: The amount of isorhamnetin glycosides loaded in the fiber was dependent to the concentration of OFI powder, solvent and amount of gelatin. The highest content of isorhamnetin glycosides was obtained using 12 g of OFI powder with acetic acid 80% as solvent (251.03 µg Iso Eq. / g Fiber). The 47.15% of the loaded isorhamnetin glycosides was released to the medium after the first 4 h, achieving a complete release of the isorhamnetin glycosides after 12 h. The isorhamnetin glycosides loaded fibers proved to be stable under physiological conditions during 48 h. In this period, the fibers were completely degraded due to its biocompatible characteristics. **Conclusion**: The results suggest that the crosslinked gelatin fibers loaded with the isorhamnetin glycosides from OFI can be used successfully as a controlled release system of bioactive compounds.

Actinomycetes from natural areas with promoting growth plant capacity

Blanca Estela Gómez Luna; Rafael Alejandro Veloz García; Juan Carlos Ramírez Granados; César Díaz Pérez, Adan Topiltzin Morales Vargas, Laura Mejia Teniente.
University of Guanajuato, Department of Agroindustrial Engineering, Celaya, C.P. 38060; be.gomez@ugto.mx, bgomezl2000@yahoo.com.mx

Introduction.
The hill Culiacan was declared as a Protected Natural Area in the category of sustainable use area and in soil exist benefic bacterias named plant growth promoting bacteria and are a group of bacteria that inhabit the root of the plants and soil attached to it, this space is known as rhizosphere (Cassán et al, 2009).
This group of bacteria provides benefits to plants through several mechanisms: N_2 fixation, phytohormone production, solubilization of phosphates, synthesis of enzymes such as ACC deaminase that reduces ethylene levels, biological control, production of siderophores, antibiotics, activation of the induced systemic response and production of lytic enzymes (Glick 1995). The products generated by the different mechanisms, have direct and indirect effects on the plant in the development and growth, such as: improvement in germination, greater development of the root, stems, leaves and fruits or defense against phytopathogenic organisms (Glick, 1995, Dobbelaere et al., 2003; Esquivel-Cote et al., 2013). In this work, we seek to isolate soil bacteria from protected natural areas and use their biotechnological potential in plant growth.
Methods
Bacteria were isolated in selective media for ACC deaminase activity. These bacteria tested their production capacity cellulases, chitinases and siderophores in sective media. Also antibiosis activity against phytopathogenic fungi was carried out.
Results.
From 20 strains there were production of siderofors in 10, production of cellulases in 16 and production of chitinases in 16 strains. Most bacteria showed inhibition and three strains with great inhibition.
Conclusions.
We were able to appreciate the beneficial effect of bacterial isolates from soils of Protected Natural Areas in diverse microbial capacity. These results indicate the biotechnological potential of natural resources and from these sites bacteria can be isolated that help preserve these ecosystems, but it is also feasible to apply to plants of agronomic interest.

***Key words**: actinomycetes, soil, biotecnology aplications*

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Relevance of Tal2a, a putative LysM effector from *Trichoderma atroviride,* on its activity as mycoparasite and plant endosymbiont

Sandra M. Gómez-Méndez, Yordan J. Romero-Contreras, Paulina Guzmán-Guzmán and Vianey Olmedo-Monfil.

Laboratorio de Interacción Planta-Microorganismo, Departamento de Biología, DCNyE Universidad de Guanajuato. Noria Alta S/N Col. Noria Alta, C.P. 36050, Guanajuato, Gto., México vg.olmedo@ugto.mx

The interaction of plants with fungi is one of the most studied biological processes, since it has a direct impact on agricultural productivity. The main focus has been to understand the events that lead to the development of diseases caused by phytopathogens. It is known that during plant-fungus interactions, the plant defense system is activated, mediated by the recognition of Molecular Patterns Associated to Microorganisms (MAMPs) which leads to the syntheses of compounds like lignin, phytoalexins and PR-proteins, including chitinases, glucanases, and proteases, which have a key role in defense mechanisms, preventing the establishment of the fungus in the plant. Successful fungal pathogens have developed mechanisms that involve effectors in many cases, to counteract and/or evade plant defense responses.

Effectors have the ability to modify the structure and function of the host cell, facilitating the infection. In particular, LysM effectors have been related to events on which the perception of the fungus by the plant is prevented through various mechanisms, among them cell wall protection, chitinases inactivation and hijacking of chitin that work as a MAMP. *Trichoderma* species are some of the beneficial fungi present in the rhizosphere; are known as mycoparasites with antagonistic activity against several phytopathogens, additionally some species have been reported as plant endophytes with the ability to promote growth, development and the enhancement of the plant defense response.

However, there is still little information about the molecular mechanisms that facilitate *Trichoderma* - plant interaction. We are interested in studying the early events of this process, starting from the recognition of the fungus by the plant. Previously we reported six effector candidates that encode proteins with LysM domains in *T. atroviride*. Out of these genes, four have shown an increase in their expression patterns during the interaction with *A. thaliana*, for example *Tal6* and *Tal2a*. In the case of the Tal6 product we have confirmed its function as an effector, protecting the hyphae from hydrolytic activities and probably also by of chitin hijacking. Tal6 participates in the antagonistic capacity of *Trichoderma* and on the other hand, enhances the vigor of tomato plants to confront the phytopathogen *Rhizoctonia solani*.

In order to determine the relevance of *Tal2a*, the other differentially expressed LysM gene from *T. atroviride*, we are generating overexpressing strains to analyze the impact of this putative effector in both, mycoparasitic activity and its capacity as a plant endosymbiont.

Keywords: *Trichoderma*, Effector, Plant-fungus interaction, LysM

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"Degradation in hypersaline conditions of aromatic polycyclic hydrocarbons and pharmaceutical compounds by the halophilic strains *Aspergillus sydowii*-like H1 and *Aspergillus destrudens* EXF-10411"

Deborah González Abradelo, María del Rayo Sanchez Carbente, Jorge Luis Folch-Mallol, Ramón Alberto Batista García.
debugonzalez@gmail.com / Research Center for Cell Dynamics, University of the State Morelos.

Abstract

Polycyclic aromatic hydrocarbons (HPAs) are liposoluble substances that are formed because of combustion, the treatment of residual carbon, among other causes. On the other hand, pharmaceutical compounds are bioactive substances with a wide environmental distribution because of their incentive and extensive use, even indiscriminate. Both HPAs and pharmaceutical compounds are defined as priority environmental pollutants with the greatest biological impact, due to their disrupting, carcinogenic and mutagenic effects that they cause in living systems, and in their relationships with other living systems and the environment. In recent years, there is growing interest in the use of biotechnology with the aim of restoring ecosystems contaminated with xenobiotics. Bioremediation is based on the use of living organisms or their metabolic products to remove and / or partially or totally degrade xenobiotics present in the environment, and thus reduce their concentration and toxicity. The bacteria have been extensively studied according to their potential for the removal of environmental contaminants, including the two groups mentioned above. However, fungi have been studied to a lesser extent despite their extraordinary potential for the removal and degradation of HPAs and pharmaceutical compounds. In the literature there are practically no reports on the potential of extremophile fungi for the removal of contaminants, although many environments contaminated with HPAs and pharmaceutical compounds have extreme physical-chemical characteristics, such as high salinity. These peculiarities constitute an additional challenge for bioremediation strategies, and demand the use of extremophile organisms. The aim of the present work is: To evaluate, in hypersaline conditions, in both synthetic media and wastewater, the removal of aromatic polycyclic hydrocarbons and pharmaceutical compounds, by the halophilic strains *Aspergillus sydowii*-like H1 and *Aspergillus destruens* EXF-10411. While *Aspergillus sydowii*-like H1 is a moderate halophilic fungus, *Aspergillus destrudens* EXF-10411 is an obligate halophilic fungus, being one of the few ascomycetes that require fiercely high concentrations of NaCl for growth. In this work the enzymatic activities (laccase, esterase and peroxidase, during the removal of contaminants were characterized, and finally, the removal of HPAs and pharmaceutical compounds in mixtures in the presence of hypersaline conditions (> 1M NaCl) was evaluated. post-treatment was evaluated through germination trials of cucumber seeds (*Cucumis sativus*) Both strains proved to be effective in the removal of HPAs and pharmaceutical compounds in the presence of hypersaline conditions, this being the first study investigating the removal of these xenobiotics in halophilic conditions.

"Design and production of CRISPR-dCas9 and dCas12a dimers for the formation of nanostructurs with DNA"

Itzel G. González Carmona, Carlos Calcines Cruz, Lucila A. Arroyo Rabasa, Armando Hernández García
Instituto de Química, UNAM
Circuito Ext. C. U. Del. Coyoacán 04510 México, Cd. Mx. Apartado postal 70-213 Teléfono: (55) 56224548 Correo: armandohg@iquimica.unam.mx

CRISPR-Cas is a defense mechanism used by bacteria to degrade exogenous genetic material. It consists of a protein component (for example, Cas9 or Cas12a) that binds first to a guiding RNA, which directs the protein to the DNA sequence to be recognized. **(1)** This mechanism has been used for genetic editing, however there are still other fields in which it could be used favorably, as for example, in the manufacture of DNA nanostructures with proteins. Recently, the use of TAL effector protein dimers in the formation of nanostructures with DNA was demonstrated.**(2)** The disadvantage of the use of these proteins to obtain a specific structure, is that is required produce a lot of proteins to recognize and bind the various binding sites on the DNA, which takes time and is expensive. On the other hand, CRISPR-Cas proteins offer the possibility of varying the sequence that they unite on DNA in an easier, faster and more precise way, only by varying the RNA that guides them.

This project proposes to use dCas9 and dCas12 protein dimers (inactivated versions of its nuclease activity) for binds to DNA in different positions and forms nanostructures. This would open new perspectives for the use of the CRISPR-Cas system, which to date has only been used to edit genomes, but never for nanotechnological purposes. The dimerization will be achieved by fusing the dCas9 and dCas12 proteins to a system, highly characterized and used, that utilizes FRB-FKBP dimerization domains induced chemically by the addition of Rapamycin. **(3)**

In this way it is expected to obtain the dCas9 and dCas12 proteins bound to an FRB or FKBP domain for subsequent polymerization in the presence of Rapamycin, thus achieving the dimer: dCas9-dCas9, dCas12-dCas12, dCas9-dCas12, dCas12-dCas9 and determine its DNA binding, for the formation of nanostructures with DNA. This will be done using standard molecular biology and sequencing techniques, recombinant expression in E. coli and purification by chromatography with a nickel affinity column. In addition to checking the weight of the proteins with an acrylamide gel and MALDI-TOF. Follow by dynamic light scattering to measure the increase in the sizes of the proteins in solution. And determine the binding of dimerized proteins on a DNA template by EMSA.

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Production in E. coli and IMAC purification of the C-terminal region of the *Schizosaccharomyces pombe* FXNA peptidase

González Esparragoza Dalia[1,2], Herrera Camacho Irma[2], Rosas Murrieta Nora[2], Luna Félix[3].
[1]Maestría en Ciencias Químicas, BUAP.
[2]Lab. Bioquímica y Biología Molecular, Centro de Química del Instituto de Ciencias BUAP.
[3]Laboratorio de Neuroendocrinología, Facultad de Ciencias Químicas, BUAP.

Edificio IC7, Ciudad Universitaria-BUAP. 72570 Puebla Pue. Tel. 229-5500 ext. 7295. e-mail: daly.qfb04@gmail.com

SUMMARY

In mammal cells, the FXNA peptidase also named endoplasmic reticulum metallopeptidase 1, is a multi-pass peptidase membrane protein localized in the endoplasmic reticulum (ER) membrane of the rat granulosa cells and required for the organization between somatic cells and oocytes into discrete follicular structures. Recently, the FXNA peptidase was recognized in various human cancer cells as potential oncogene involved in endoplasmic reticulum stress and unfolded protein responses (UPR). UPR is a best characterized stress response mechanism present in all eukaryotic cells, a pathway very important for to modulate the capacity of folding proteins in the lumen of the ER. Although the information has been provided on its role in mechanisms of proliferation, migration and invasiveness of the cancer cells and normal cells, the proteolytic mechanism of the FXNA peptidase is still unknown. Previously, we identified and sequenced the FXNA mRNA in the fission yeast *Schizosaccharomyces pombe (S. pombe)* that encoded a putative FXNA peptidase homologous called "FxnaSp", a protein with low similarity with rat FXNA peptidase (29%). In *S. pombe*, the mRNA transcription level is not modified throughout the growth phases of the yeast. However, there were other peptidases that did not allow us to characterizer and proof of identity the FxnaSp putative peptidase. In the present work the aim was to produce recombinant FxnaSp protein in *E. coli* and to get anti-FxnaSp antibodies for hydrophilic domain located in the C-terminal region of the peptidase, also to identify and lock up in *S. pombe* cells. A fragment of 709 bp was amplified by PCR from DNA chromosome of the *S. pombe* 972 h^- strain, it which corresponds to the region of the ORF that encodes for the hydrophilic domain located in FxnaSp C-terminal region containing the BamHI and PstI restriction sites at the ends. The PCR product was purified and ligated in to the vector pJET1.2/Blunt for its amplification in the Top10 strain of *E. coli*. Then, the fragment (FxnaSP-Cterm) was subcloned by enzymatic digestion into the pQE-80L vector and it was transformed in *E. coli* TOP10. Afterward, the recombinant vector was purified and the construction was confirmed by enzymatic digestion and sequenced, the BL21 strain of *E. coli* was transformed with the recombinant vector for expression. Subsequently, the optimal conditions of expression, production and purification were established. The FxnaSp-Cterm protein fragment as obtained from the inclusion bodies after of the induction process with IPTG of the E. coli BL21 strain. The inclusion bodies were solubilized in ionic sarkosyl detergent (5%), and the protein fragment purified using immobilized metal affinity chromatography–(Agarose-NTA-Ni). Each step of the protein purification was observed by western-blot using anti-histidine antibodies. After that, we established the optimal binding and elution conditions with different concentrations of imidazole. Lastly, we obtained a protein fragment of approximately 26.9 kDa corresponding to FxnaSp-Cterm with top quality and harvest.

Characterization of protease activities in mid-gut of *Chrysoperla carnea* larvae

Claudia de la paz González García[3], Javier Castillo Castillo[1,] Domancar Orona Tamayo[2], Dalia Rodríguez Ríos[2], Manuel Darío Salas Araiza[3], Alejandro Hernández Morales[1], Gertrud Lund[2], José Eleazar Barboza Corona[3], Gustavo Hernández Guzmán[3*]
1) Universidad Autónoma de San Luís Potosí, Unidad Zona Huasteca
2) CINVESTAV Unidad Irapuato
3) Universidad de Guanajuato, División de Ciencias de la Vida, km 9.0 Carr. Irapuato-León, Irapuato, Guanajuato, México. CP 36500. +52-462-6241889 Ext. 5234. gustavohdz@ugto.mx

Chrysoperla carnea (Stephens) Neuroptera: Chrysopidae, is a widely used insect in integrated pest management programs because of voracity and easy rearing protocols. *C. carnea* larvae inject a liquid secretion to prey in order to paralyze and digest internal tissues. Currently, secretion composition is not defined and our research group is dedicated to study enzymatic fraction by using non-denaturing gels coupled to zymogram analysis in order to investigate protease activities contained in mid-gut protein crude extracts. By using specific protease inhibitors we reported Serin-, Cystein- and Metallo-proteases in crude extracts. Most abundant protease activities are related to Trypsin-like proteases. 2D nondenaturing gel analysis of crude extracts coupled to zymograms showed specific spots and coalescent zones containing several spots showing protease activities.We selected specific spots and were submited to protein mass spectrometry analysis. RAW data were compared to selected databases and interestingly showed up similarity to different spider, cobra and scorpion proteases and venoms.
Work is underway to clone those protease like gene coding sequences. Final objective of our research is to characterize and describe at molecular detail *C. carnea* digestive secretion by transcriptome sequencing and bioinformatic data analysis.

Isolation of halotolerant bacteria, biosurfactant producer

Jorge Gracida, Daniela Ugalde-Sánchez, José Ramírez-Ramírez, Aldo Amaro-Reyes
Universidad Autónoma de Querétaro. Facultad de Química. Cerro de las Campanas S/N, Centro Universitario, 76010 Santiago de Querétaro, Qro. 5567867162 gracidaj@netscape.net

The biosurfactants are amphipathic molecules composed mainly of structures such as peptides, glycopeptides, fatty acids and phospholipids. They have the ability to reduce the surface tension at the interface liquid-liquid, or liquid-gas. Moreover, these molecules are produced and secreted by diverse microorganisms and some plants. These are found mainly in sites contaminated with hydrocarbons as well as in places associated with osmotic stress conditions.
In this work, six different bacteria were isolated from Zapotitlan Salinas Puebla and one identified as *Bacillus* sp (16S gene). The soil of this place have 3 % (w/w) of NaCl. Different Carbon Nitrogen (C/N) ratio were tested trying to increase the biosurfactan productivity. We observed that the marine broth with a C/N of 16 was the best culture condition to promove the biosurfactant production, allow to diminish the surface tension of the culture media at 30 mN/m (40 h). *Bacillus* sp was able to growth in a marine medium with hight salt concentration present (120 g/L).

MANUFACTURE OF DNA MICROARRAY FOR IDENTIFICATION OF TRANSGENIC ORGANISMS IN MAIZE.

Simón Guzmán León, José Luis Santillán Torres, Lorena Chávez González, Jorge Ramírez Salcedo.
Unidad de Microarreglos de DNA, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México. Circuito exterior, Ciudad Universitaria, Coyoacán, A. P. 70-242; México, D. F. 04360 México, Tel: 56225753.
http://microarrays.ifc.unam.mx/
sguzman@ifc.unam.mx

The main objective of the DNA Microarray Unit of UNAM is the manufacture of DNA microarrays of different biological models, with this experience it has been possible to manufacture and design microarrays that identify,simultaneously, enterobacteria in food. It has also been possible to standardize microarrays that simultaneously identify transgenic events in the corn plant.

Currently the production of corn is in high demand in Mexico and worldwide so the contribution of transgenic plants in this crop has been important.Therefore the competent authorities responsible for the detection of these transgenic organisms also have a high demand. It is for this reason that DNA microarrays, being a massive tool, it is feasible to use them multiple ways in order to simultaneously detect the transgenic events reported for Maize. For these reasons, the DNA Microarray Unit of UNAM has designed a prototype chip to detect up to now 13 transgenic events for Maize but simultaneously and in a multiple format, that is, on a chip, up to 16 different samples of Corn plants can be processed and thus check the presence / absence of said transgenic events. This identification is carried out in a maximum of 24 hours.

This chip is intended to be a tool capable of identifying the authorized transgenic events in corn or in products derived from these GMOs, in order to speed up the identification process. The tool is not intended to replace existing methods, but rather aims to contribute to the rapid and reliable analysis required to ensure the quality of food and the safety of it.

Caffeic Acid Production from Coumaric Acid in a Recombinant *Escherichia coli* Strain.

Georgina Hernández Chávez, Ana Gabriela Hernández López, Ramón de Anda Herrera, Luz María Martínez Mejía, Francisco Bolívar Zapata and Guillermo Gosset Lagarda.
Instituto de Biotecnología, UNAM, Departamento de Ingeniería Celular y Biocatálisis. Av Universidad 2001, Chamilpa. Cuernavaca, Morelos 62210, Méxicoe-mail: gosset@ibt.unam.mx

Caffeic acid (CA) is a phenolic acid frequently found in small amounts within diverse food sources. Its multiple nutritional properties, such as antioxidant, anti-inflammatory or anticarcinogenic has attracted interest in pharmaceutical and cosmetic industries. Because of this, microbial production of CA has acquired crescent interest as a biotechnological alternative (Furuya et al. 2012).

The present study is focused on determination of the conditions necessary for microbial production of CA in 250 ml flasks with 50 ml of M9 minimal salts medium, using coumaric acid as precursor and glycerol 10 g/L as carbon and energy source.

For this study, we employed W3110 *Escherichia coli* strain, overexpressing the genes for the enzyme 4-hydroxyphenyl acetate 3 hydroxylase (4HP3H) (Muñoz et al., 2011). We studied the effect of Isopropyl-β-D-1-thiogalactopyranoside (IPTG) as the promoter inducer, and of the CA precursor (coumaric acid) concentration, on the production of CA (Fig 1).

Under the described conditions, with 0.1 M IPTG, we were able to produce 2 g/l of CA from 2 g/l p-coumaric acid in 10 g/l glycerol, which represents a 100% substrate conversion. This recombinant *E. coli* strain that produces CA can be employed as a platform for the production of various plant phenylpropanoids by metabolic engineering.

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Cultivation of the cyanobacterium *Desertifilum* sp. for the production of biomass and C-Phycocyanin

Ingrid Hernández Martínez[1], Laura González Reséndiz, León Sánchez García, Izlia Arroyo Maya[2], Marcia Morales Ibarria[2*]
[1]Maestría en Ciencias Naturales e Ingeniería, Universidad Autónoma Metropolitana-Cuajimalpa
[2]Departamento de Procesos y Tecnología; Universidad Autónoma Metropolitana-Cuajimalpa, Av. Vasco de Quiroga 4871. C.P. 05348, Mexico City. Phone: +52 55 5814 6500, Ext.:3807
Corresponding author: mmorales@correo.cua.uam.mx

Keywords: Desertifilum sp.*, cell growth, extraction, C-Phycocyanin*

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C-Phycocyanin is a blue protein-pigment that is part of the photosynthetic complex that captures light in cyanobacteria and red algae, it has been used as a natural colorant in food and cosmetics and has potential for nutraceutical and biomedical applications due to its antioxidant, anti-inflammatory, anticancer, antiviral and neuro-protective properties (1). C-Phycocyanin can be extracted from many species of cyanobacteria including *Spirulina platensis*, however, it requires large amounts of salts to grow. The aim of this study was to evaluate the growth and potential of the fresh water cyanobacterium *Desertifilum* sp. for the production of C-Phycocyanin.

Desertifilum sp. was cultivated in a 0.9 L stirred tank-photobioreactor operated in batch mode. Mineral medium BG-11 was used and incubated at 35 °C and irradiance of 40 µmol m^{-2} s^{-1}. Two experiments were performed, one without pH control and another with pH control by pure CO_2 injection on demand. The biomass concentration was measured by dry weight and subsequently the cyanobacterial cells were harvested by centrifugation and the phycobiliproteins were extracted by repeated freeze-thaw cycles of the cells. The obtained crude extract was purified through a fractionation with ammonium sulfate and then subjected to dialysis, the entire process was carried out in dark at 4 °C (2).

It was observed the maximum biomass (X_{max}) was 0.59 g L^{-1}, a productivity (P) of 65 mg L^{-1} d^{-1} with a specific growth rate (µ) of 0.26 d^{-1} under normal conditions of culture without pH control; and values were 0.92 g L^{-1}, 100 mg L^{-1} d^{-1} and 0.30 d^{-1} for X_{max}, P and µ, respectively when photobioreactor had pH control. The purity ratio of C-Phycocyanin, A_{620}/A_{280}, was determined by spectrophotometry, where A_{620} is the maximum absorption of phycocyanin and A_{280} is the absorption of proteins in general (2). The A_{620}/A_{280} was 3 after dialysis process with a concentration of 2.4 mg mL^{-1}.

Cultivation of *Desertifilum* sp. with pH control had a better growth and a higher biomass productivity, in addition the purity of the C-Phycocyanin have promising applications for the food sector. This is the first work evaluating the growth of this cyanobacterium.

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Evaluation of *ptsG* and *galR* deletions on glucose transport and catabolic repression in a lactogenic strain of *Escherichia coli*

Tlakaelel Akolmiztli Hernández Ríos, Estefanía Sierra Ibarra and Alfredo Martínez Jiménez
Universidad Nacional Autónoma de México – Instituto de Biotecnología. Cuernavaca, Morelos, 62210, México.
tlakael@ibt.unam.mx

Most bacteria have a hierarchical consumption of sugars present in the environment, which allows adapting to different carbon sources available in the environment. This regulatory mechanism is known as catabolic repression or repression by carbon catabolite (CCR). In *Escherichia coli*, regulation of CCR depends on the concentration levels of the phosphorylated enzyme IIA from the phosphotransferase transport system (PTS). Phosphoenolpyruvate (PEP) provides the energy to this system transferring the phosphate group, through a cascade of phospho-carrier proteins, to glucose and allowing its internalization. There are alternatives to PTS, by which glucose is internalized in the cell. Consumption of this sugar depends on two systems involved in the transport of galactose. One of these systems is through the low-affinity galactose proton-simporter, encoded by *galP*, which expression is repressed by GalR.

An *E. coli* strain that preferentially produces D-lactic acid (D-LA) from pentoses and hexoses was obtained using metabolic engineering tools and adaptive evolution. This evolved D-lactogenic *E. coli* strain, named JU15, was previously sequenced and the following genotype was revealed: *ΔpflB, ΔadhE, ΔfrdA, ΔxylFGH, gatC S184L, ΔmidarpA, Δreg 27.3 kb*, being the galactitol mutant (GatC S184L) responsible for efficient xylose transport. Results showed that strain JU15 produces D-LA with high yield and productivity in mineral media in pH-controlled, non-aerated batch mini-fermenters. Strain JU15 showed sequential carbon source utilization: first glucose and then xylose.

The *ptsG* gene, which encodes the EIIBC component of the PTS transport system, was deleted to avoid catabolic repression of xylose from glucose. The effect of the *ptsG* deletion on lactogenic JU15 was also assessed with mineral media in pH-controlled batch mini-fermenters. Cultures performed with glucose and strain JU15 Δ*ptsG* showed a specific growth rate (μ) of 0.12 h^{-1} and a specific glucose consumption rate (qGLC) of 2.06 g glucose/(gDCW.h), which represents a decrease of 65% and 75%, respectively, compared to the values obtained with strain JU15. Through adaptive evolution, a spontaneous variant of JU15 Δ*ptsG* (JU15 Δ*ptsG-variant*) was obtained, in which μ increased two-fold and qGLC 2.14-fold in comparison to JU15 Δ*ptsG*. To evaluate the effect of the galactose transport system on glucose consumption the *galR* gene was deleted on strains JU15 Δ*ptsG* and JU15 Δ*ptsG-variant*, to allow the constitute expression of the galactose transporter. This strategy allowed to increase 25 % the μ and 15% the qGLC when compared JU15 Δ*ptsG*-Δ*galR* to cultivations with JU15 Δ*ptsG*. Unexpectedly, deletion of *galR* in JU15 Δ*ptsG-variant* strain caused a decrease on μ (33 %) and qGLC (38.5 %). This study reveals that GalR has other regulatory functions. When xylose was used with glucose (20 g/L each), in strain JU15 Δ*ptsG*, both sugars were consumed simultaneously, and the volumetric consumption of xylose was higher than glucose; hence the phenomenon of catabolic repression was abolished with the lactogenic strains under non-aerated conditions.

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Leucine aminopeptidase yspII from *Schizosaccharomyces pombe* involved in mitosis.Revertant of the mutant strain (4XΔape2).

Irma Herrera Camacho[1], Getulio Flores Tlalpa[1,2], Lourdes Millán Pérez Peña[1], Nora Hilda Rosas Murrieta[1].
[1] Laboratorio de Bioquímica y Biología Molecular, Centro de Química del Instituto de Ciencias, BUAP.
[2]Maestría en Ciencias Químicas, área de Bioquímica y Biología Molecular, BUAP.
Edificio IC7, Ciudad Universitaria- Benemérita Universidad Autónoma de Puebla. 72570 Puebla Pue. Tel. 229-5500 ext. 7295. e-mail: irma.herrera@correo.buap.mx

SUMMARY
Leucine aminopeptidases (LAPs) are metallopeptidases from M1 and M17 families with diverse functions such as virulence factor, metabolic regulators, meiosis and progression of cancer, among others. In 2007, LAP-yspII was described in Schizosaccharomyces pombe, a manganese-dependent homohexameric basic cytoplasmic enzyme of 320 kDa, encoded by the *ape2* gen. Previous studies in our laboratory, upon interrupting the *ape2* gene, we observed a decrease in the growth rate in the logarithmic stage as well as a significant decrease in cell viability in the final logarithmic phase, observing significant changes in its cellular morphology, finding anucleated cells or with an unequal distribution of the chromosomal material, which indicates that there are problems in cell division. The objective of this work was to reverse the abnormal phenotypic effects caused by the interruption of the *ape2* gene in the mutant strain, by reincorporating the complete gene into the mutant strain and thus confirming that LAPyspII plays an important role in mitosis.
The construction of the reversal vector pRSP-ape2 was obtained, which includes the promoter, UTRs and CDS of the *ape2* gene, elements necessary for the LAP yspII to be expressed and regulated by its native promoter. This vector was incorporated into the mutant strain 4XΔape2. The revertant strain obtained "4XΔape2Rev", presents the same levels of enzymatic activity of the LAPyspII of the control strain 4X. The kinetic behavior of the revertant strain was recovered, the kinetic parameters being such as maximum speed and doubling time statistically equal to the control strain. Cell viability was recovered in the revertant strain. Regarding the distribution of the genetic material during cell division, stains (for nucleus with DAPI and for membrane lipids with FM 4-64) of cells from the control, mutant and revertant strains in different stages of growth were carried out 32 to 44 hrs (final logarithmic phase and stationary start). In the mutant strain, an unequal distribution of the genetic material, an amorphous compaction of the chromosomes and anucleated cells, and the appearance of cytoplasmic agglomerations that had not been described before were observed again. In the revertant strain, no evidence of these morphological traits was found, behaving in a very similar way to the control strain, cylindrical cells with uniform and well-defined nuclei. Based on the results, it was confirmed that the phenotypic effects in the mutant strain are due to the absence of LAP-yspII, which supports the idea that this enzyme participates in the control of mitosis and the segregation of genetic material.

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Effect of nutritional and physicochemical factors on the dimorphic transition and production of α-amylase in the yeast *Candida wangnamkhiaoensis*

Raziel Arturo Jiménez Nava[1*], Zahuiti Fabiola Hernández Montañez[1], Griselda Ma. Chávez Camarillo[1]
Laboratorio de Microbiología Industrial, Escuela Nacional de Ciencias Biológicas. IPN, Prol. Carpio y Plan de Ayala S/N, Santo Tomás, Miguel Hidalgo. C.P. 11340, CDMX. Tel 5729-6000 ext. 62382
*Author of the work's presentation.
Contact: arturojimeneznava@yahoo.com.mx

Candida wangnamkhiaoensis (previously called *Wickerhamia* sp.) is a dimorphic yeast that produce a thermostable α-amylase of 55.3 kDa, with an optimal pH of 6.0 in yeast-like phase.

Several studies related to the production systems show that in a repeated fed-batch culture the enzyme productivity is 7000% higher than in batch culture.

In the presence of glucose, the synthesis of the α-amylase is partially repressed but not inhibited, even at the concentration of 30 g/L. The starch showed to be more efficient than maltose for the induction of the enzyme.

Due the biotechnological potential of α-amylase, in this work we evaluated the effect of different carbon and nitrogen sources, as well as pH and oxygen concentration influence in the dimorphic transition of this yeast, both in solid and liquid culture.

Most relevant results show that glucose, maltose, glutamate and ammonium sulfate are the carbon and nitrogen sources that more stimulate the mycelial growth in *C. wangnamkhiaoensis* in solid and liquid culture media.

The pH effect evidenced that at values under 7.0, the mycelial formation was improved. In contrast, higher values promote the switch to the yeast-like phase. Also the mycelial phase was improved by increasing the oxygen concentration in the culture media using a bubble column reactor.

The results of the present work contribute to the knowledge of the physiology of *C. wangnamkhiaoensis*, since there is no information about it. Moreover, we give a guide for control and maintenance of the yeast-like phase, since in this phase the mayor productivity of the α-amylase is obtained.

SYNTHESIS OF CAFFEIC ACID USING VARIABLES OF CYTOCHROME BM3

Jorge Luis Jiménez Niebla, Gloria Saab Rincón*.
Instituto de biotecnología, UNAM.
Av. Universidad 2001, Chamilpa, 62210 Cuernavaca, Morelos. +52 (777) 329-1640
*gsaab@ibt.unam.mx

Caffeic acid is an important metabolite for the plant that is used as a precursor in the phenylpropanoid pathway. This compound has proven to have important applications for the pharmaceutical industry, for that reason different biosynthetic strategies have been tried to find suitable ways of obtaining this molecule. Its precursor, p-coumaric acid is a compound that is widely distributed in plant tissues as a product or in its conjugated form. In contrast to caffeic acid, it is a relatively inexpensive reagent that is commercially available. The conversion of p-coumaric acid to caffeic acid depends on a hydroxylation at C3 of the aromatic ring; This reaction is catalyzed by cytochrome p450 in plants: However, eukaryotic cytochromes are anchored to a membrane, making their expression in other organisms difficult, besides of their dependence on the activity of a reductase to regenerate its catalytic site.
The cytochrome P450 of Bacillus megaterium (CYT BM3) is a soluble and selfsufficient bacterial cytochrome that uses straight-chain fatty acids as substrates. CYT BM3 has been widely used as a biocatalyst for a wide variety of substrates with different chemical properties. It has been seen that the CYT BM3 is not able to use small organic molecules naturally, so we seek to create an intelligent library using bioinformatics tools for the analysis of its structure and a high throughput screening method to select and characterize mutants capable to hydroxylate p-coumaric acid efficiently.

The analysis of the structure of the catalytic site of CYT BM3 help us to select important residues involved in the molecular recognition and catalysis to design a library of mutants that we predict can modify the enzyme specificity to carry out the hydroxylation reaction of caffeic acid.

Isolation of three new novel nor-triterpenes with anti-inflammatory activity from a population of the Mexican species *Galphimia glauca* Cav. grown on the state of Morelos

Eleazar León-Álvarez [*1], Alexandre Cardoso-Taketa[1], Pablo Núñez-Aragón[2], María Luisa Villarreal[1]
[1]Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos, Avenida Universidad 1001, Col. Chamilpa, Cuernavaca, Morelos México. C.P. 62209. 2Facultad de Farmacia, Universidad Autónoma del Estado de Morelos [1], alvarez-leon2@hotmail.com

Key words: *Galphimia glauca*, 1H NMR, triterpenes, anti-inflammatory activity

The species *Galphimia glauca* Cav. (Malpighiaceae) is a medicinal plant endemic to Mexico and used to treat nervous system disorders [1]. Studies of the metabolomic of *G. glauca* collected from different localities of the Mexican Republic, demonstrated that the secondary metabolites content varies according to the area of collection. However, in all the studied populations anti-inflammatory activity using the ear edema model in mice induced by 12-O-tetradecanoylphorbol (TPA), was observed. In three populations collected in the states of Morelos and Jalisco, some compounds that revealed in a blue coloration using Thin Layer Chromatography (TLC) were observed [2]. These compounds had not been identified before, so the objective of the present investigation was based in the purification and structural characterization of these metabolites in individuals of *G. glauca* that were collected in Tepoztlán Morelos, México.

The plant material was collected in the months of March and July of 2017, then the maceration technique was implemented in methanol using the stems and the leaves of the dry and grounded plant. The methanolic extract was fractionated in different open column chromatography systems using silica gel 60 of size 30-70 µm, and a mobile phase $CHCl_3$: A_cOEt (1: 4). The ^{1}H and ^{13}C NMR spectra of the pure compound were obtained, as well as their 2D correlation records. The *in vitro*, anti-inflammatory activity of the compounds was evaluated using the murine macrophages RAW 264.7 cell line. Additionally, *in vivo* tests were performed on CD1 mice edema induced by TPA.

Through TLC, a compound (denominated as ELA-0) with an Rf = 0.40 and revealing blue, was observed by the use of vanillin-H_2SO_4 in the methanolic extract of the stems of *G. glauca*, then purified through open column chromatography and sent to NMR testing. The resultes showed the presence of a new *nor*-triterpene (C_{30} H_{44} O_6). In the leaves of *G. glauca* two compounds that revealed in blue color (named ELA-1 and ELA-2) with Rfs = 0.41 and 0.68 respectively were isolated. ELA-1 presented a CH_3 COO-, while ELA-2 exhibited two CH_3 COO- groups in their chemical structure making them different to ELA-0. So far, the results of the *in vitro* anti-inflammatory activity of ELA-1 and ELA-2 showed a better anti-inflammatory activity with an IC_{50} of 18.92 and 16.67 µg / mL respectively, compared with the complete methanolic leaves extract with an IC_{50} of 44.89 µg / ml. The results of the *in vivo* tests showed that the methanolic extract with a n = 6 at a dose of 0.3-3 mg / ear presented an IC_{50} of 1.94 mg / ear, while for ELA-2 at a dose of 0.1- 1.3 mg / ear the IC_{50} value was 0.87 mg / ear, indicating that ELA-2 exhibited a better anti-inflammatory activity compared to the methanolic extract. Currently, evaluations of the *in vivo* anti-inflammatory action of ELA-0 and ELA-1 are ongoing.

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Callus induction and phytochemical profile of *Jatropha curcas* L.

Gerardo Leyva Padrón[1], Crescencio Bazaldúa Muñoz[1], Silvia Evangelista Lozano[1], Pablo Emilio Vanegas Espinoza[1] y Alma Angélica Del Villas Martínez[1]; 1Instituto Politécnico Nacional, Centro de Desarrollo de Productos Bióticos, Departamento de Biotecnología, San Isidro, Yautepec, Morelos, C.P. 62731, gleyvapadron@gmail.com

Jatropha curcas L. is a plant that belongs to Euphorbiaceae family, historically this plant has been associated to multiple uses such as ground recovery, and processing of feed, food, pesticides, cosmetics, and anticancer medicine. This plant accumulates cyclic diterpenes, whith biological activities, as those derived from lathyrane or tigliane. It has been reported *in vitro* propagation protocols for *J. curcas* L. on both toxic and non-toxic variety, using different explants on media supplied with contrasting concentrations and combinations of plant growth regulators. Callus are cell clusters in an undifferentiated state, which can be induced to a cell re-differentiation process through the addition of plant growth regulators, as well as to produce secondary metabolites of industrial interest. Cell culture is an alternative for the production of compounds of interest, an example of a high-value drug produced from plant cell cultures is paclitaxel, an anti-cancer drug extracted from *Taxus brevifolia*. Unfortunately, the dedifferentiation of plant cells during callus induction often results in a decrease in their capacity to synthetize compounds normally produced in plant. The aim of this work was to know the variation on the callus accumulation of diterpenes. A comprehensive study on the phytochemical contents of the hydro-ethanolic (80:20) and hexane:isopropanol extracts from leaves and callus on both toxic and non-toxic varieties of *Jatropha curcas* L. was conduced. Cellular dedifferentiation was induced in petiole explants, which were desinfected (ethanol 80% and sodium hypochlorite 2%), cultivated on MS medium added with naphthalene acetic acid (NAA) and benzylaminopurine (BAP) (1.5 mg/L each). Two extraction methods (ethanol 80% and hexane:isopropanol) were tested. Cell cultures were dehydrated at 50°C. Extracts were analyzed by HPTLC using chloroform-methanol (94:6) as mobile phase, and revealed with ceric sulfate. Taxol was utilized as reference standard. NAA and BAP played an important role in the formation of callus obtained from petiole explants. Explant dedifferentiation was evident after 7 days of culture, and 96.6% of explants dedifferentiation was achieved at day 21. The HPTLC plate revealed was observed at 254 and 366 nm. Several spots with similar RF's to the taxol (0.49) were observed. This result allows supposing the presence of cyclic diterpene compounds similar to taxol in leaves and callus on both toxic and non-toxic varieties of *J. curcas* L.

Physiological and molecular characterization of *Saccharomyces cerevisiae* strains for Tequila production.

Araceli López Andrade, Naurú Idalia Vargas Maya, Gloria Angélica González Hernández, Adriana García Tapia, Israel Enrique Padilla Guerrero and Juan Carlos Torres Guzmán*.
Departamento de Biología. División de Ciencias Naturales y Exactas. Universidad de Guanajuato, Campus Guanajuato, Noria Alta s/n, Colonia Noria Alta. Guanajuato, Gto., México. C.P. 36050. Tel. (473) 7320006 ext. 8160. Email: torguz@ugto.mx

In Mexico, non-distilled and distilled alcoholic beverages produced from different *Agave* species had been of great importance since pre-Hispanic times. Tequila is an alcoholic beverage distilled from *Agave tequilana* Weber Blue Variety must. During the fermentation step, a variety of yeast species have been found. However, at the end of the fermentation, *Saccharomyces cerevisiae* became the dominant yeast (Oliveras *et al*., 2008).

S. cerevisiae is traditionally ethanol-producer; however, the fermentation efficiency changes depending on the strain´s origin. It has been evaluated the behavior of yeasts of different origins during fermentation. Changes in the behavior of the yeasts can be attributed to genome rearrangements. Gene duplication is an important evolutionary mechanism; however, duplication not only refers to the individual gene but large chromosomal segments and even whole-genome duplication can happen (it is thought to have occurred 100 million years ago in *S. cerevisiae*, Wolfe et al., 1997). Duplication of large segments of the genome is common in yeasts, and it had been found a correlation between functions specific of duplicated genes and the environment from which the strains were isolated (Ames *et al.,* 2010). Analysis of chromosomal profiles of *S. cerevisiae* industrial strains had shown that the yeast genome could undergo extensive changes. Wine yeast strains have a marked chromosomal DNA polymorphism, and they have "hybrid" chromosomes (Bidenne et al., 1991). We have a collection of different *S. cerevisiae* strains that were isolated from *Agave* must and used in the industry for Tequila production. These yeast strains exhibited different behavior when are exposed to distinct stress conditions and during fermentation of *A. tequilana* must. We think that their genomes have rearrangements in consequence of the stress conditions from the environment to which they have been exposed. From the *S. cerevisiae* LABGENMOLHO2 strain that produces Tequila with desirable aromatic characteristics, using methods of combination with the strain LABGENMOH01; which has a high fermentation efficiency and is capable of making fermentations of high-gravity (high concentration of sugars). A new strain was isolated (T40) with combined characteristics of both yeasts, its molecular characterization and its main physiological features were carried out, as well as fermentations in *Agave* must to analyze the main volatile compounds produced.

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Modulation of the activity and expression of the peroxidase ZmPrx35 from insect-resistant maize endosperms (*Zea mays L.;* P84C2R) in response to mechanical and insect damage.

Laura Margarita López-Castillo[1], María Fernanda Díaz-Flores-Rivera[1], Alán González-Leyzaola[1], Mariana Marrero-Bretado[1], Robert Winkler[2] and Silverio García-Lara [1]
[1] Escuela de Ingeniería y Ciencias, Tecnológico de Monterrey. Av. Eugenio Garza Sada 2501 Sur. C.P. 64849. Monterrey, N.L.
[2]Departamento de Biotecnología y Bioquímica. Cinvestav Unidad Irapuato. Km. Km. 9.6 Libramiento Norte Carr. Irapuato-León. C.P. 36824 Irapuato, Gto.
E-mail: lmlopez@itesm.mx

Maize (*Zea mays L.*) is the largest staple crop produced worldwide. Mexico has been considered as the origin and diversification center of this cereal. Fifty-nine native varieties have been identified in this country. Most of them are still cultivated by smallholders with limited access to land and modern production resources. Postharvest primary insect pests, especially the maize weevil (*Sitophilus zeamais)* and the large grain borer (*Prostephanus truncatus)* cause grain losses during storage up to 40% of total production, affecting mainly to smallholders from developing countries. Actual alternatives for pest management, such as the implementation of hermetic storage structures and the application of chemical insecticides are often unaffordable to smallholders. In consequence, modern breeding programs have endeavored to develop insect-resistant varieties. However, the knowledge of mechanisms and bases of natural resistance is still limited.

In recent years, phenolic acids and endosperm peroxidases have been identified as chemical bases of insect resistance in maize kernels. However, their role is still discussed. Studying the highly insect-resistant *Z. mays* variety P84c3 was found that more than 90% of the total POD activity was provided by a single enzyme, identified as B6T173_MAIZE (or ZmPrx35), a class III peroxidase. The role of this novel enzyme in the insect resistance remains unknown.

The aim of this work was to identify the contribution of ZmPrx35 to the mechanisms and bases of insect resistance displayed by kernels from some maize varieties and landraces. Interactions between insect-resistant endosperms (P84C2R) and the storage pests *S. zeamais* and *P. truncatus* demonstrated that ZmPrx35 activity and expression is modulated by mechanical and insect damage, suggesting a contribution of this enzyme in the mechanism of antixenosis (repellence) for *S. zeamais*. In the case of *P. truncatus*, this pest inhibited kernel POD activity, suggesting pest adaptation to POD/phenolic defence mechanism.

Our findings will contribute to the screening for insect resistant maize varieties and will support marker-assisted breeding and the identification of natural insect repellents

Generating a diagnostic method for differentiation of all four serotypes of dengue virus by PCR, using samples Tabasco State

López Mondragón Daniela Alejandra[1], Santos Fernández Ingrid Araceli[1], Landa Pineda César Manuel[1,2], e-mail: mondragonale18@gmail.com, [1]Lic. Químico Farmacéutico Biólogo. [2]Maestría y Doctorado en Química Aplicada. [3]Laboratorio de biología molecular y Farmacogenómica. Universidad Juárez Autónoma de Tabasco. División Académica de Ciencias Básicas. Carretera Cunduacán-Jalpa KM. 1 Col. La Esmeralda CP. 86690 Cunduacán, Tabasco, México.

Dengue fever is a viral disease with a higher prevalence; virus belongs to the Flaviviridae family, genus Flavivirus or group B of Arboviruses. *Aedes aegypti* mosquitoes are the primarily vector. The clinical features that causes this fever dengue can be confused with others virus of the same family and present similar symptoms. Dengue virus has a small genome RNA and to date has been described four serotypes (DENV-1, DENV-2, DENV-3 & DENV-4) with clinical relevance. The development disease, commonly involves just a one serotype, however more than one may be present, which is called coinfection. The virus needs favorable environmental conditions to spread and survive, such as those that present in tropical and subtropical regions; Tabasco state has similar conditions that influence at proliferation for this vector, this can be spread and transmission the diseases caused by arboviruses.

The fever dengue is high incidence, and according to data recorded by the World Health Organization (WHO), it is estimated that in 2010 there were 96 million apparent infections by dengue worldwide, of which 14% of the cases (9-18 million) belong to the American continent and more than half of these cases occurred mainly in the countries of Brazil and Mexico. According to the 2014 reports of the Panamerican Health Organization, in Mexico, between 100,000 and 200,000 cases of dengue were reported. According to data recorded by the Ministry of Health in 2016, the state of Tabasco registered an incidence of dengue virus of approximately 19%. This state occupies the 5th place in the national in the number of confirmed cases. The DENV-2 has highest frequency and only has been reported three of four serotypes present into state.

The differential diagnosis of this virus is highly important for the appropriate medical treatment can be defined, without the danger of aggravating the pathology.

This work has as main objective to differentiate the dengue virus serotypes from the rest of the arboviruses using technique of endpoint PCR, using targeted primers that allow us to quickly and accurately diagnose the etiological agent.

For this, were analyzed complete genomic sequences by multiple alignments of the different serotypes of the dengue virus. The optimal PCR conditions were standardized and were used blood samples the patients with a clinical diagnosis like to fever dengue.

Efficient pyruvate production from glucose and xylose using metabolically engineered *Escherichia coli* and limited oxygen transfer conditions

Mauricio López Portillo Masson, Alejandra Vargas-Tah, Georgina Hernández Chávez, Estefanía Sierra Ibarra and Alfredo Martínez Jiménez
Universidad Nacional Autónoma de México - Instituto de Biotecnología, UNAM. Av. Universidad 2001, Chamilpa, 62210 Cuernavaca, Morelos, malopo@ibt.unam.mx

Pyruvate is a main intermediate in the metabolism of organisms and has become an important commodity molecule and building block for the synthesis of many drugs and agrochemicals as well as in the food industry and cancer research; hence its production using fermentation technologies needs to be studied in detail.
In this work, a fermentation process for pyruvate production with a high yield and productivity, using xylose and/or glucose as carbon sources, was designed based on the use of metabolic engineering and bioprocess tools. The strain MG1665 of *Escherichia coli* was metabolic engineered to generate a non-fermentative strain with improved capacity for xylose metabolism, the strain was called AV13, **(***ΔpflB, ΔadhE, ΔfrdA, Δldh, ΔxylFGH, Δreg 27.3 kb, gatC S184L, ΔmidarpA ΔmgsA ΔpoxB Δack-pta).* As all fermentative pathways were removed (to produce formate, ethanol, succinate, lactate and acetate), the strain AV13 is unable to grow under non-aerated conditions. To oxidize the reducing power (NADH+H) limited amounts of oxygen must be provided and pyruvate should be produced and secreted. Therefore, the volumetric productivity of pyruvate using AV13 can be maximized under oxygen limiting condition, wherein a low oxygen transfer would avoid the generation of high biomass concentrations without decreasing the catabolism of sugars to pyruvate.
E. coli AV13 was evaluated in 1 and 3-liter fermenters using mineral media with glucose, xylose or a mixture of both (total concentration of 74 g/L), at 37 °C and controlling the pH at 6.5 by automatic additions of KOH. The aeration rate was maintained at 0.55 vvm and the oxygen mass transfer, measured as the volumetric oxygen transfer coefficient (k_La) was modified ranging the stirring speed.
Using the above-mentioned strategy, we were able to decrease the time needed to achieve sugar depletion (74 g/L) from 120 hours (k_La =40 h^{-1}) to 26 hours (k_La =145 h^{-1}), while increasing the biomass concentration only from 2 to 6.13 gDCW/L. The pyruvate concentration increased from 40 to 62.50 g/L and the volumetric productivity from 1.48 to 2.40 g/(L.h). Higher k_La values (>200 h^{-1}) were unfavorable for pyruvate generation because significant amount of biomass and CO_2 were produced. The genetic background of the strain (*ΔxylFGH, gatC S184L*) enabled the co-consumption of xylose and glucose without the drawback of a diauxic growth, therefore, a mixture of both carbon sources can be used to produce pyruvate, for example from laboratory sugars or hydrolysates of lignocellulosic residues such as corn stover.

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Analysis of molecular dynamics in modified sugar transporters for bioethanol production

Aline López-Vargas, Carlos Regalado, Andrés Cruz, Aldo Amaro y Jorge Gracida
Autonomous University of Querétaro
Cerro de las Campanas S/N Col. Las Campanas Querétaro, Qro. C:P: 76010,
gracidaj@netscape.net

The production of second generation bioethanol requires industrial conversion processes that are economical and sustainable, in addition to take advantage of renewables as lignocellulosic biomass. Modified strains of Saccharomyes cerevisiae have been developed with robust metabolisms that allow the increase of bioethanol yields and make them tolerant to high levels of inhibitors. Although these mentioned improvements, the ability to introduce different sugars is still inefficient [1]. The yeast S. cerevisiae has a family of 18 different hexose transporters (Hxt), which mainly regulate the entry of glucose, therefore, no other type of sugars have been used. Four aminoacids were identified, which constitute the YYXT/P motif in the Hxt and which allowed to direct in transport to other types of sugars such as xylose [2,3]. Using a molecular dynamics of proteins analysis, we aim to determine specific mutations in motif YYXT/P to control the transport of glucose and xylose. The results are key for the development of the strains that will adapt to the sugar profile present in raw material. This will allow to increase ethanol production by using fruit waste to obtain this biofuel.

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Design and construct of two modules to detect *Listeria monocytogenes* using *Escherichia coli and Bacillus subtilis* as chassis

Celia Monserrat Luna-Castro[1], *Luz Edith Casados-Vázquez*[1,2], *José Eleazar Barboza Corona*[1,2*]
[1]Universidad de Guanajuato, Campus Irapuato-Salamanca (CIS), División de Ciencias de la Vida, Posgrado en Biociencias. [2]Universidad de Guanajuato, Campus Irapuato-Salamanca (CIS), División de Ciencias de la Vida, Departamento de Alimenbtos. Ex-Hacienda El Copal km. 9, carretera Irapuato-Silao; A.P. 311, C.P: 36500, Irapuato, Gto. México. Cel. 4621659334, Correos electrónicos: monlu_46@hotmail.com, josebar@ugto.mx*

Listeria monocytogenes (Lm) is an important food-borne human pathogen able to survive in a wide range of environmental conditions (Freitag et al., 2009). This Gram-positive microorganism harbors a peptide-based two-component quorum-sensing system known as Agr quorum sensing (agrQS) which regulate the expression of many virulence genes and biofilm formation and is crucial for controlling the microbial motile and sessile lifestyle. This system, is regulated by the PII promoter that control the expression of *agrB, agrD, agrC*, and *agrA*. Briefly, AgrD is the pro-peptide that forms the quorum sensing molecule autoinducer peptide (AIP), while AgrB is a transmembrane protein responsible for cyclizing AgrD, generating AIP. The remaining members, AgrC and AgrA, form the two-component system responsible for AIP detection and response, respectively. Upon binding to AIP, AgrC a transmembrane histidine kinase receptor, facilitates the phosphorylation of the transcriptional activator AgrA. The detection of AIP by AgrC activates the phosphorylation of AgrA that turns on expression regulated by pII. In this work, we designed and assembled the modules pcspA-agrA-agrC-pII-rfp and pspac-agrA-agrC-pII-rfp and then transform *Escherichia coli* and *Bacillus subtilis,* respectively. Our strategy consists that once Lm release AIP, this will be detected by AgrC and then the AgrA is going to phosphorylate and activate the pII promoter that regulates the expression of the red fluorescent protein (RFP) protein. Currently we are assessing the effectiveness of both biosensors.

Keywords: *Listeria monocytogenes*, biosensor, quorum sensing.

Micropropagation from nodal segments of *Vaccinium corymbosum*

[1]María del Mar Machado Achirica, [2]Alejandra Flores Martínez, [2]María Isabel Ibarra Pérez, [1]Osiel Salvador Recoder Meléndez, [1]Lisset Herrera Isidrón,
[1]Instituto Politécnico Nacional, Unidad Profesional Interdisciplinaria de Ingeniería Campus Guanajuato. CP36275. Silao de la Victoria, Gto. 2TREO BERRY FARM, Tepatitlán, Jal. México
Corresponding author: mar.machado.95@gmail.com, lherrerai@ipn.mx

The blueberry (*Vaccinium corymbosum* L.) is a commercially important crop worldwide and their fruits are excellent source of antioxidant foods. Tissue culture propagation has been established in order to reach genetic uniformity in the planting materials and to overcome problems such as the variations in fruit characteristics. In the present work, the micropropagation of blueberry plants was carried out. Shoots tips from commercial genotypes were taken as explants and disinfected in a commercial solution NaCl (1.0, 1.5 o 3.0%) plus Tween (0.1%) followed by a treatment in 70% ethanol. The best result was obtained when stems were treated with 1.5% NaCl solution during 5 min. In order to determine the growth, Woody Plant Medium (WPM) was added with Zeatin or N6- (delta 2-isopentyl) - Adenine (2IP) in combination with Indoleacetic acid (AIA). In terms of sprout micropropagation the WPM medium supplemented with Zeatin (1.44 mg/ L) and AIA (0.01 mg/L) elicited a greater than threefold increase in the number of shoots and nodes generated
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Induction of hairy roots by three strains of *Agrobacterium rhizogenes* on *Agastache mexicana*

Angélica Lucía Martínez Aguilar[1], Gabriela Carmona Castro[1], Susana Valencia Díaz[1], Samuel Enoch Estrada Soto[2], Irene de la Concepción Perea Arango[1].
[1]Centro de Investigación en Biotecnología. Laboratorio de Botánica Estructural.
[2]Facultad de Farmacia. Laboratorio de Farmacognosia. Universidad Autónoma del Estado de Morelos. Av. Universidad 1001. Colonia Chamilpa, C.P. 62209, Cuernavaca, Morelos.
email: iperea@uaem.mx

Agastache mexicana (Kunth) Lint & Epling (Lamiaceae) is an aromatic and perennial herb widely distributed in southern North America. *A. mexicana* exhibits antioxidant, antinociceptive, antiinflammatory, spasmolytic and sedative activities and it is also used in curing stomachache, nervous disorders and wounds. Phytochemical studies of aerial parts extracts performed until now have documented the presence of some flavonoids, terpenoids, alkanes and phenols. Among flavonoids, tilianin is a special object of interest from pharmaceutical and cosmetic point view. Tilianin, a glycosidase flavone isolated from *A. mexicana,* to possess antihypertensive and anxiolytic activities at non-toxic concentrations.

In vitro root culture has become an alternative method for the production of secondary metabolites with pharmaceutical and cosmetic interest. Genetic transformation with *Agrobacterium rhizogenes* is a means for hairy root induction, which have been used for the production of secondary metabolites (e.g., flavonoids). The goal of research was, to investigate the induction and growth of hairy roots from *Agastache mexicana* using *Agrobacterium rhizogenes*.

Three strains of *Agrobacterium rhizogenes* (ATCC15834, K599 and A4) were tested to determine their effects on the induction of hairy roots on explants from cotyledons, hypocotyls and leaves and in *in vitro* plants. Explants did not produce roots. However, hairy roots were induced directly from the wounds of stems *in vitro* seedlings from 4 weeks old grown in Murashige and Skoog semi solid medium using *A. rhizogenes* strains ATCC15834, K599 and A4 strains. The hairy root lines were confirmed by molecular characterization, but these have been selected for their rapid growth rate and high production of tilianin. Among the three strains of *A. rhizogenes*, ATCC15834 was found to be the most efficient in transformation and initiation of hairy roots.

The information obtained from this promising hairy root cultures may contribute to a potent biological model for enhancing the production of potentially active molecules, such as tilianin.

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Removal of chromium VI by fungal isolated associated to lichens

Fernando Martínez-Armenta[1]; Oscar-Guadalupe Castillo-Martínez [1]; César Álvarez-Mejía[2]; Divanery Rodríguez-Gómez[1], Varinia López-Ramírez[1].
[1]Instituto Tecnológico Superior de Irapuato. Coordinación de Ingeniería Bioquímica. Carretera Irapuato - Silao km 12.5, C.P. 36821. 2Instituto Tecnológico Superior de Abasolo. Blvd. Cuitzeo de los Naranjos #401, Col. Cuitzeo de los Naranjos, C.P 36976. valopez@itesi.edu.mx, Tel. 462-6067900 Ext. 141.

Chromium is a toxic and carcinogenic heavy metal that prevails in wastewater. In nature exists in trivalent and hexavalent form. Cr (VI) is more toxic due to its solubility. It has been shown that fungi associated to lichens have the ability to tolerate and remove chromium. In this project we evaluated the growth and percentage of removal of Cr (VI) by a fungus isolated from lichens, M008, individually and in a consortium with another fungal isolate. Two chromium-tolerant fungal isolates evaluated previously by our group (M008 and ORM18) were inoculated in Petri dishes with Sabouraud Dextrose Agar (SDA) with and without chromium (3 and 5mM). We evaluated the type of interaction, mutualistic or antagonist, between the two fungal isolated. The isolate M008 was inoculated in Sabouraud Dextrose Broth (SDB) with Chromium in concentrations 800 µM, 3 mM and 5 mM, and we measured biomass growth by dry weight technique, every 48 hours by 16 days. The controls were SDB without chromium and SDB with chromium (800 µM - 5 mM), with inoculum and without inoculum respectively. The two chrome-tolerant fungal isolates were inoculated in SDB with chromium (3 and 5 mM) individually and in a consortium, we measured biomass growth, by dry weight technique, at 48 and 240 hours. The controls were SDB without chromium, inoculated individually and in consortium, and SDB with chromium (3 and 5 mM) without inoculum. The percentage of removal of chromium was determined by atomic absorption technique. The interaction between the fungal isolates M008 and ORM18 was mutualistic and the fungal isolate M008 tolerates and is able to grow in concentrations of 800 µM, 3mM and 5mM, however, as the concentration increases, its growth decreases. The percentage removal of chromium (3 and 5 mM) will be estimated. Our results show that the fungal isolate M008 is able to tolerate chromium at 800 µM, 3 mM and 5mM concentrations, its capabilities of Cr(VI) tolerate point to a dynamic strategy, and the description of it is undergo.

UTMBioLAB: Molecular-Biotechnological Diagnostic

Eber Diego Martínez Correa.[1], MDC. Martha Amalia Avalos Medina.[1], DC. Mario Javier Gutiérrez Fernández.[1] Universidad Tecnológica de Morelia. 1 Av. Vicepresidente Pino Suarez 750, Ciudad. Industrial, 58200. mgutierrez@utmorelia.edu.mx

Abstract The following work was developed with the aim of being able to carry out the projection and management of a lab facility for the Biotechnology Engineering career of the Technological University of Morelia, mainly to understand and carry out techniques that are focused on the molecular diagnosis of infectious diseases, for an early diagnosis and impact on public health.

The diagnosis as such, is the act of knowing the nature, in this case of a disease through the symptoms and signs and involves making decisions that should define the progress of the care of an individual, this being the reason why a diagnosis is fundamental both for who describes it and where it is carried out. Currently molecular biology laboratories are a constant application, revolutionizing the health system, leading biomedical research and innovating medical treatments. To be more specific, the molecular diagnosis includes the bases to carry out molecular biology techniques with a biomedical approach, from one or several specific DNA, RNA or Protein sequences.

One of the greatest contributions in the medical industry in the field of molecular biology was the discovery and implementation of a well-known and currently used technique called Real Time Polymerase Chain Reaction (RT-PCR), which in broad terms allows us to perform amplifications of specific DNA sequences, delimited by sequences mostly known as Primers.

Infectious diseases make up approximately 50% of the molecular diagnostic techniques currently available. The reason is that pathogens are difficult to detect by classical microbiology, and considering that molecular biology techniques only merit DNA sequences, it is not mandatory to have a microorganism in the sample, but only its genetic material.

Therefore Biotechnology students can use these techniques for the optimal development and innovation of future molecular diagnostic techniques by manipulating various microorganisms, of which at this stage we focus as an object of research with *Mycobacterium tuberculosis* (tuberculosis), *Treponema pallidum (*syphilis*) and alphavirus (*Chikungunya*).*

Biochemical study of the production of melanin in the mutant ΔPMA1 of *Ustilago maydis* and its potential use in bioremediation.

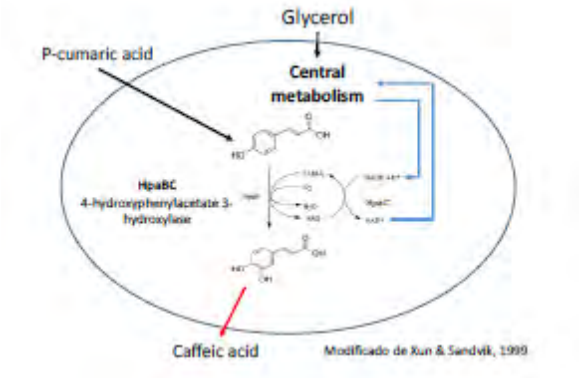
Martínez López Cinthya, Hernandez Morfin Katia Daniela, Zepeda Giraud Luis Fernando, Olicón Hernandez Dario, Oscar flores Herrera, Guerra Sánchez María Guadalupe.
Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional. Prolongación de Carpio y Plan de Ayala s/n, Col. Santo Tómas, C.P 11340, Delegación Miguel Hidalgo, Ciudad de México. Tel. 57296000 ext. 62339. Correspondence e-mail: lupegs@hotmail.com

The elimination of soil, air and water pollutants generated by heavy metals, pesticides, dyes and industrial chemical compounds represent a challenge for environmental biotechnology. *Ustilago maydis* is a phytopathogenic fungus, which causes corn charcoal known as huitlacoche. In our working group, we obtained a mutant of the enzyme H + ATPase, ΔPMA1. This mutation resulted in the accumulation of a large amount of melanin. Melanins are natural pigments found in animals, plants and microorganisms, they are produced from the oxidation and polymerization of phenolic compounds and synthesized by the tyrosinase enzyme. The melanins have interesting physicochemical properties as a broad spectrum of light absorption and chelating capacity. In view of the chemical diversity of melanins, there is a growing interest and search to develop new industrial applications for their use. Therefore, in the present work, it is proposed to use the mutant strain (ΔPMA1) as biosorbent agent of heavy metals and dyes. To achieve this objective, the growth of the wildtype (FB2) and mutant strain was determined, reaching the exponential phase in the first 12 h of the culture with rates of 0.281 ± 0.004 and 0.221 ± 0.003 h-1 with duplication times of 2.46 and 3.13 h for FB2 and ΔPma1, respectively. The mutant (ΔPMA1) showed higher tyrosinase activity compared to the wildtype strain. The pigment extracted was purified and analyzed by U.V-VIS and infrared, comparing the obtained spectra with the synthetic melanin (sigma). The spectroscopic profile was an eumenlanin. Finally, to determine the dye biosorption, different concentrations of methylene blue were used in minimal media and the absorbance of the supernatant was determined. So far, the data obtained show that the *Ustilago maydis* ΔPMA1 mutant has a higher production of melanin and a better biosorption of the dye.

HETEROLOGOUS PRODUCTION OF CAFFEIC ACID IN RECOMBINANT *Escherichia coli*

Luz Maria Martínez Mejía, Jorge Sánchez Andrade, Georgina Hernández Chávez, Guillermo Gosset Lagarda*.
Departamento de Ingeniería Celular y Biocatálisis, IBt/ UNAM.
Av. Universidad # 2001, Col. Chamilpa, Cuernavaca, Mor. C.P. 62210.
*tel.: +52 (777) 329-1601, gosset@ibt.unam.mx

Caffeic acid is a secondary metabolite present in all plants because it is an intermediate in the biosynthesis of lignin, one of the main forms of biomass. Potential pharmacological effects such as antioxidant, anti-inflammatory and anticancer of caffeic acid make it a compound of industrial interest. Caffeic acid is obtained by extraction from vegetable sources employing chemical or enzymatic hydrolysis. These methods represent a high economic and energy cost and are also hostile to the environment. A promising and convenient alternative is to produce caffeic acid through biotechnological processes expressing in microorganisms the phenylpropanoids pathway, which starts from the aromatic amino acids A second alternative strategy is to supplement the culture medium with the phenylpropanoid precursor p-coumaric acid.
In this work, employing genetic engineering tools, three strains of recombinant *Escherichia coli* were generated using two expression vectors: pTrc99A or pCDFDUET in BL21DE3 strain. Cloning strategies, employing either the Trc or the T7 promoter, were evaluated to express the *hpaBC* genes from *Escherichia coli* W, which code for the enzyme 4-hydroxyphenylacetate 3-hydroxylase (EC 1.14.13.3), this enzyme can hydroxylate p-coumaric acid to caffeic acid:



The growth and production kinetics of caffeic acid in M9 minimal medium supplemented with p-coumaric acid as precursor of the synthesis of caffeic acid and glycerol as carbon source were evaluated. As a result, it was established that of the three strains generated, the strain *Escherichia coli* BL21DE3/pTrc*hpaBC* has the best capacity to produce caffeic acid from p-coumaric acid in minimal medium M9 with glycerol as carbon source reaching titers up to 2.0 g/L.

Expression and purification of recombinant glucose oxidase in *Pichia pastoris* and evaluation of cytotoxic and anticancer activity *in vitro*

Authors: Evelyn Martínez Mora, María del Rosario González González, Xristo Zárate Kalfópulos, Eder Ubaldo Arredondo Espinoza, Isaías Balderas Rentería
Universidad Autónoma de Nuevo León, Laboratorio de Ingeniería Genética y Genómica, Facultad de Ciencias Químicas.
Av. Universidad S/N, Colonia Universitaria, San Nicolás, Nuevo León, México. Tel: 83294000 ext 3435 and 6221; e-mail: isaias.balderasrn@uanl.edu.mx

Cancer is a broad group of diseases that can affect any part of the body. One of its characteristics is the rapid multiplication of abnormal cells that extend beyond their limits and can induce metastasis. It is the second cause of death in the world, with a total of 8.8 million deaths in 2015. Among the main treatments is chemotherapy, in which it has been observed that cancer cells develop resistance to multiple drugs, thus decreasing the effectiveness of the treatments. Therefore, it is necessary to improve and develop new therapeutic agents that attend this condition. It has been found that reactive oxygen species, such as H_2O_2, in high concentrations can cause cell cytotoxicity, being even higher in cancer cells. One of the H_2O_2-producing enzymes is glucose oxidase, which in the presence of a glucose molecule and an O_2 molecule forms H_2O_2 and gluconic acid.
In this work the extracellular expression of the recombinant glucose oxidase enzyme was carried out in the eukaryotic expression system *Pichia pastoris* SMD1168, through the modification and optimization of the *gox* gene of *Aspergillus niger* to improve its expression in yeast and its purification. In addition, the secretion signal of the α-factor of *Saccharomyces cerivisiae* was added; this gene was inserted into a vector for intracellular expression pPIC3.5K (Invitrogen). The insertion of the gene in the genome of the yeast was confirmed by PCR and by analysis with restriction enzymes. The extracellular expression of the enzyme was corroborated by SDS-PAGE, with a size between 63 kDa and 100 kDa and it was purified by anion exchange chromatography. The enzymatic activity of glucose oxidase was determined using horseradish peroxidase and o-dianisidine, which it exhibited a lower specific activity compared to the commercial enzyme but, the recombinant glucose oxidase showed greater cytotoxic and anticancer activity *in vitro* in Vero and SiHa cell lines.

Effect of the *SNF1* deletion in the glycolytic pathway of *Saccharomyces cerevisiae* grown at 1% glucose

Cecilia Martinez-Ortiz, Miguel David Duffo-Hurtado and Luis Alberto Madrigal-Perez
Laboratorio de Biotecnología Microbiana, Instituto Tecnológico Superior de Ciudad Hidalgo, Ciudad Hidalgo, Michoacán, 61100, México. Email: lmadrigal@itsch.edu.mx

The alcoholic fermentation is an amphibolic process that allows the formation of ATP at substrate level, keeps active the glycolysis through re-oxidation of NAD^+, and generate metabolites of biotechnological interest. *Saccharomyces cerevisiae* is a positive Crabtree, which performs the alcoholic fermentation in oxygen presence, increasing the glycolytic flux accompanied by repression of respiration. However, the molecular mechanism of the Crabtree effect is still not clear. The sucrose non-fermenting protein-1 (Snf1p) is a cellular energy sensor that modulates the switch between respiration and alcoholic fermentation. Therefore, Snf1p could be a key piece in the molecular puzzle of the alcoholic fermentation related to the Crabtree effect. For this reason, this work aimed to determine whether the deletion in *SNF1* gene affects the alcoholic fermentation at 1% glucose. For this purpose, the BY4742 genetic background was used and its mutant in the *SNF1* gene. In this way, it was found: 1) that the absence of *SNF1* gene affects growth. 2) A decrease in extracellular acidification rate in *SNF1* mutant. 3) NADH/NAD^+ ratio does not have any change in the strains. However, the amount NAD(P)H showed an increase in *SNF1* mutant. 4) Transcription levels of *HXK2* gene displayed an increase in *SNF1* mutant; while transcription levels of *PFK1* gene decrease. 5) The basal respiration and the maximum respiratory capacity diminished in *SNF1* mutant. All data indicate that deletion in *SNF1* gene favors flux of carbonated species to the cycle of pentoses phosphate probably by a transcriptional regulation of glycolysis, not observing an effect in the alcoholic fermentation.

Isolation and *in vitro* evaluation of peptides for their potential use as immunogens against *Anaplasma marginale*

Julián Martínez Salgado[3], Jesús Francisco Preciado[1], Lidia González Morales[2], Rosa Estela Quiroz Castañeda[1], Mayra Elizeth Cobaxin Cárdenas[1], Eduardo Vergara Rivera[4], Sergio D. Rodríguez Camarillo[1], Itzel Amaro Estrada[1*]
[1]CENID Parasitología Veterinaria (INIFAP), [2]Centro de Investigación en Biotecnología (UAEM), [3]Maestría en Biotecnología (CEIB - UAEM), [4]Licenciatura en biología (Facultad de Ciencias Biológicas, UAEM).
*Centro Nacional de Investigación Disciplinaria en Parasitología Veterinaria, INIFAP Carr. Fed. Cuernavaca-Cuautla 8534, Col. Progreso, Jiutepec, Mor., C.P. 62550. (777) 3192860 ext.127 amaro.itzel@inifap.gob.mx; amaro.estrada@gmail.com

Keywords: *Anaplasma marginale*, Immunoglobulins, Phage display, IgG2.

Anaplasma marginale is an obligate intraerythrocytic Gram-negative bacterium from the order Rickettsiales, it presents high antigenic diversity among geographical isolates, a characteristic that is being studied for its importance in the development of control strategies. In cattle, can cause bovine anaplasmosis, which is an infectious disease but non-contagious, characterized by fever, anemia, general weakness, abortions, depression, anorexia, weight loss, pale mucous membranes, decreased milk production and death in some cases. In Mexico there is not available commercial vaccine and the economic losses due to the bovine anaplasmosis are still in force.

Otherwise, the importance of the production of immunoglobulins of subclass G2 (IgG2) against bovine anaplasmosis has been proven. Cattle protected against homologous challenge show a response that it involves IgG2 but not IgG1 production. Therefore, IgG2 present in serum of protected bovine can be useful for to identify relevant ligands. Screening of random peptides libraries displayed on phage surface is a powerful tool for studying protein interactions. It has allowed the identification of ligands recognized by polyclonal antiserums and it has been successful for the identification of relevant epitopes of different viral proteins using serum from infected patients.

Due to the need to have an immunogen to contend with *A. marginale* infection, we performed three rounds of biopanning using purified IgG2 or blood sera of immunized bovines against *A. marginale* as ligands of the random peptide library Ph.D. ™ -C7C. We generated a collection of recognized peptides tested by ELISA employing direct coating with molecules used in panning. We selected some candidates and their DNA sequence were determinate; we identified seven peptides sequences deduced from the nucleotide composition. The selected phage-peptides were evaluated by ELISA performed with IgG2 purified of three different immune sera. In this approach, we differentiated three phage-peptides for their ligand binding, specificity, affinity and stability properties. However, in the case of the development of vaccines or diagnostic reagents, it is important to evaluate the immunogenic and antigenic properties of the peptides.

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Degradation of diesel and waste oil by *Pseudomonas spp*. isolated from contaminated soil

Gabriela Estefanía Moran Medina*, Andrea Daher Rodríguez*, Irais Castillo Maldonado† y José Antonio Martínez Villalba*
**Departamento de Ingeniería Ambiental. Universidad Iberoamericana Torreón, Calzada Iberoamericana 2255. Col Ejido la Unión. Torreón, Coahuila, México. CP 27420. Tel. (871) 705 1019 correo: jose.martinez@iberotorreon.edu.mx*
† Departamento de Bioquímica del centro de investigación Biomédica. Universidad Autónoma de Coahuila, Av. Morelos 900 Oriente, Torreón, Coahuila, México. CP 27000. Tel. (871) 722 6470 Ext 110 correo: irais. castillo@uadec.edu.mx

Abstract
Nowadays, biodiversity is constantly threatened by environmental disasters. The oil spills, inadequate handling of materials and hazardous waste have become a constant contamination of soils and water bodies. A strategy to recover these contaminated sites is bioremediation by microorganisms that reduce the adverse effects of these pollutants. The objective of this study includes the isolation, characterization and identification of microorganisms with the capacity to degrade waste oil residues and diesel. The isolated strains were cultivated in MSM and Bushnel Hass enriched to 0.5, 1 and 1.5% with the contaminants as the sole carbon source. Only 3 strains were able to adapt to the contaminated medium of the 11 isolated strains. Growth kinetics were performed at different conditions (pH 3, 5 and 7) in order to monitor cell growth (600 nm) and extracellular protein production by the Bradford method every 24 hours for 15 days. Strain D2 showed the highest growth in diesel. It also produced the highest amount of extracellular proteins after the tenth day (120 mg/L). On the other hand, strain D3 obtained the best performance in waste oil (OD 0.385 and 1.5%) followed by D2 at a concentration of 1.5% and OD 0.135. The optimal growth was given at pH 5 in all cases. Molecular analyzes show bacteria of the genus *Pseudomonas spp*. From this study, it is concluded that the isolated microorganisms show capacity to degrade diesel and residues of waste oil as the sole carbon source.

Key words: *Kinetics studies, diesel, waste oil, strains, growth rate*

Study on the saccharification of the lignocellulosic residue from the processing of jackfruit (artocarpus heterophyllus)

Medina-Morales José Uriel*, Leticia Mónica Sánchez-Herrera, Antonio Hidalgo-Millán, Edgar Iván Jiménez-Ruiz, Raúl Delgado-Delgado*.

Programa de Maestría en Ciencias Biológico Agropecuarias (CBAP), UAN-CA-255, UAN-CA-260; Universidad Autónoma de Nayarit, Ciudad de la Cultura "Amado Nervo" s/n, Centro, Tepic, Nay., 63155, México.*r.delgado@uan.edu.mx

Abstract
This work deal withthe hydrolysis of the lignocellulosic residue of the jackfruit with sulfuric acid at concentrations of 2, 4, 6, 8, 10% (w/w), at temperatures of 90 and 105°C for times of 30, 60, 120 and 180 min of reaction to obtain reducing sugars from lignocellulosic material of jackfruit.

Keywords
Lignocellulose, acid hydrolysis, jackfruit, reducing sugars.

Introduction
There is a great interest in the use of lignocellulosic biomass as source of reducing sugars to obtain compounds of commercial importance in the context of biorefineries (1). These sugars can be derived from lignocellulosic waste, which can be generated by activities such as agriculture. One of the sources of lignocellulosic waste, in Nayarit, is the cultivation of jackfruit, from which, approximately 40% of the fruit is discarded as waste (3), in addition, the jackfruit contains 21.29% of cellulose on a dry basis (4); therefore, the use of this material has potential to be used due to its volume of production and its possible conversion into reducing sugars. The objective of this study is to obtain reducing sugars by acid hydrolysis of the lignocellulosic residues of jackfruit.

Materials and methods

The lignocellulosic waste from the jackfruit was dried for 96 hours at 50°C and milled to a particle size of less than 1.4 mm.
Acid hydrolysis was realized using 5 grams of lignocellulosic material and 50 mL of acid were added at concentrations of 2, 4, 6, 8 and 10% of sulfuric acid. A reaction was carried out at temperatures of 90 and 105°C with residence times of 30, 60, 120 and 180 min.
The content of sugars produced were determinated by the DNS method, 0.5 mL of liquid sample of the hydrolysis were placed into a tube, 0.5 mL of DNS were added, the mixture was heated at boiling point during 5 min, 5 mL of distilled water was added and chilled for 1 min in a cold bath. The concentration of sugars was determined by spectrometry at 540 nm.

Results and discussions

Figure 1 shows the concentration of reducing sugars for the reaction conditions studied.

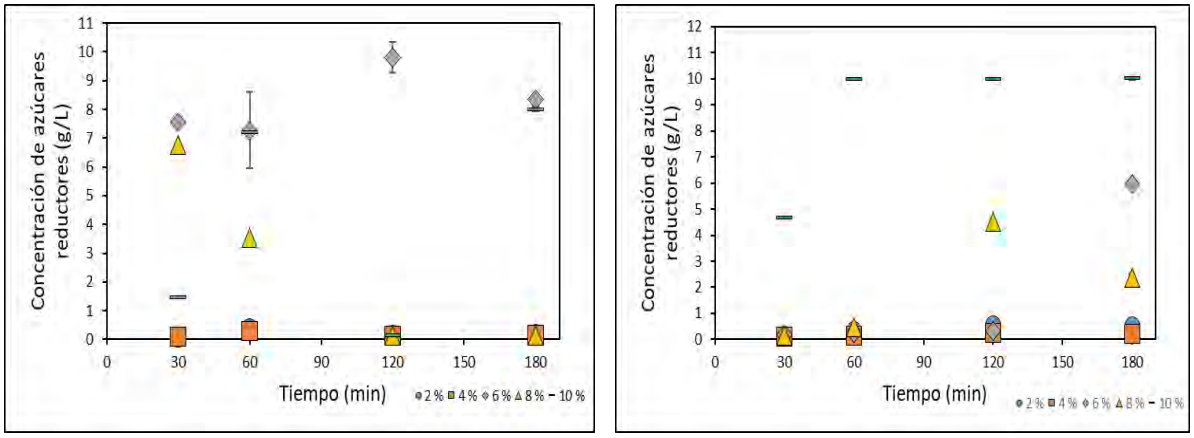

Figure 1. Results for the production of reducing sugars from the acid hydrolysis of the waste substrate of jackfruit with sulfuric acid concentrations weight in weight (w/w): a) 90°C and b) 105°C.

At 90 ° C, a maximum concentration of reducing sugars of 9.81 g/L is achieved at 120 minutes with an acid concentration of 6% (Figure 6a). Besides, at 105° C, a maximum concentration of sugars of 9.98 to 10.01 g/L was observed using 10% acid from 60 minutes with a production of 9.98 g/L holding until 120 minutes reaching 10.01 g/L at 180 minutes (figure 6b). Furthermore, it can be observed that the acid concentration of 8% does not reach the obtained responses at 6%. This can be attributed to the fact that the conditions promote more the constant of degradation rate of reducing sugars than the formation rate of reducing sugars.

Conclusions

The concentrations of 6% and 10% obtained the best responses at 90°C and 105°C respectively, in all the times. The increase and decay of concentration of reducing sugars is further appreciated, this is due to the parallel reactions of sugars which occur upon reaching a considerable concentration of pentoses and hexoses sugars that function as reactants to produce furfural and hydroxymethylfufural respectively.

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Evaluation of the phenotypic and molecular response of the interaction *Capsicum annum* L- peroxyacetic acid - geminiviruses

Mejía-Teniente Laura[1a*], Ramírez-Rangel Raymundo[1], Gómez-Luna Blanca Estela[1a], Núñez-Colín Carlos Alberto[1], Ramírez- Granados Juan Carlos[1a], Morales-Vargas Adan Topiltzin[1a], González-Chavira Mario Martin[2]

[1a]:CA. de Biotecnología, Sustentabilidad e Ingeniería. Universidad de Guanajuato, Campus Celaya-Salvatierra, DCSI-Depto. Ingeniería Agroindustrial-PE Ingeniería en Biotecnología, Av. Mutualismo Esq. Prolongación Río Lerma S/N, Celaya, Guanajuato, 38060, Mexico.
[2]: Campo Experimental Bajío, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Km. 6.5 Carretera Celaya – San Miguel de Allende. Apartado Postal 112. Celaya, Guanajuato, 38110, México.

*Corresponding author e-mail: laura.mejia@ugto.mx

Application of peroxyacetic acid in young plants of *Capsicum annuum* L. were performed, to evaluate the role of this elicitor in induced systemic resistance (ISR) leading to tolerance and / or resistance to stress biotic in plants. Peroxyacetic acid concentrations used were 80 ppm, 100 ppm, 120 ppm, 140 ppm and 160 ppm, and the viral mobility of mixed inoculations of geminiviruses in plants induced with peroxyacetic was evaluated. As well as expression of transcripts related to biotic stress and oxidative stress such as npr1, pr1 cat1, pal, were realized. Our results show that the foliar application of peroxyacetic acid elicitor in *C. annuum* L., induces
tolerant phenotype to mixed inoculations of geminivirus respect to control plants. Similarly, it was confirmed that the elicitor foliar application, induces the expression of npr1, pr1 cat1, pal genes. Finally, of the treatments applied, it is observed that the phenotypes of the concentration of 80 ppm show a greater tolerance to the appearance of characteristic symptoms generated by the mixed inoculations of geminiviruses.

Stimulation of TOR signaling pathway by the rhizobacteria *Azospirillum brasilense* in *Arabidopsis*

Manuel Méndez Gómez, Elda Castro Mercado, Ernesto García Pineda
UMSNH, Instituto de Investigaciones Químico Biológicas. Ed. A1´. Ciudad Universitaria. CP: 58000. Tel: (443) 3265788 Ext. 123. Morelia, Michoacán, México. Email: manmendezgomez@gmail.com

Key words: *Azospirillum brasilense*, plant-microorganism interaction, Target of Rapamycin

The plant growth promoting rhizobacteria (PGPR) *Azospirillum brasilese* Sp245 promotes plan growth through different interaction mechanisms. However, the plant molecular components regulated by this rhizobacteria are mostly unknown. The TOR (Target Of Rapamycin) protein is a kinase that regulates many aspects of growth and development in different organisms. TOR signaling pathway has been less studied in plants than in mammals. We studied the role of this protein during the interaction of *Azospirillum brasilense* Sp245 with *Arabidopsis thaliana*. Six days after interaction an increase in shoot and root fresh weight, lateral roots and root hairs formation, and a decrease in primary root length in *A. thaliana* was observed, associated with a reduction in cell division in the root meristem. Using the *A. thaliana TOR:GUS* line, an increase in TOR expression in the shoot, root meristem, and in the primordia of lateral roots after inoculation with the rhizobacteria was clearly distinguished. The *A. brasilense* mutant *FAJ0009*, impaired in auxin biosynthesis, was unable to activate TOR expression at the levels observed to wild type. Plant growth promoted by the rhizobacteria was inhibited in presence of the TOR inhibitor AZD-8055. In addition, *A. brasilense* was unable to stimulate plant growth in *tor-es1* plants, which decrease TOR expression in presence of estradiol. These results suggest that *A. brasilense* induce morphological changes in *A. thaliana* through TOR protein, and that the protein activation is mediated by the phytohormone auxin.

*Pv*KNOLLE: a molecular marker for the *in vivo* visualization of the cytokinesis in *Phaseolus vulgaris* roots

Elizabeth Monroy Morales, Emmanuel Ayala Guzmán, Jonathan Gilberto Cuellar Vega, Raúl Dávila Delgado and Rosana Sánchez-López. Departamento de Biología Molecular de Plantas, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Av. Universidad 2001, 62210, Cuernavaca, Morelos. Corresponding author E-mail: rosana@ibt.unam.mx

In plant cells, cytokinesis involves the formation of a cell plate (CP), a membrane-polysaccharide compartment that partitions the cytoplasm and organelles after nuclear division. The cell plate is formed *de novo* at the center of the cell-division plane as a tubulo-vesicular network that radially grows to fuse to the parental plasma membrane. It develops by the active fusion of one to another vesicle derived from Golgi/*trans*-Golgi network. Later-arriving vesicles are delivered to the margin of the growing CP. In *Arabidopsis*, this process depends on SNARE complexes involving KNOLLE, a plant cytokinesis-specific syntaxin. *At*KNOLLE expression is cell-cycle dependent, the gene is transcribed specifically in the G2 and M-phases during the cell cycle, whereas the protein is synthesized only in the M-phase. At the end of cytokinesis, *At*KNOLLE is targeted to the vacuole for degradation[1].

In order to generate a cytokinesis-specific molecular tool to easly visualize cell-division events in *P. vulgaris* roots, we have performed a search for the *PvKNOLLE* gene. In a previous work, we have demonstrated that *PvKNOLLE* promoter is active in the dividing pericycle cells, in the meristematic cells during the lateral root formation, as well as, in the cells of the root apical meristem (RAM). In this study, we have addressed the analysis of *P. vulgaris* transgenic roots bearing either the cassette prom*Pv*KNOLLE:YFP-*Pv*KNOLLE. We have confirmed that the expression of YFP-*Pv*KNOLLE in the RAM is restricted to the dividing cells, with a subcellular localization potentially associated to the cell plate. Results obtained in a similar analysis of agroinfiltrated *Nicotiana benthamiana* leaves will also be illustrated. Currently, we are performing the visualization of YFP-*Pv*KNOLLE expressed under control of the ubiquitous promoter 35S.

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Analysis of diclofenac biodegradation in a continuous bioreactor by free cells of ascomycetes fungus.

Dario Rafael Olicón-Hernández, Clementina Pozo, Jesús López-González, Elisabet Aranda.
Institute for Water Research, University of Granada, Spain. Ramón y Cajal 4, Edificio Fray Luis, Granada, 18071, Granada, Spain. Phone: 958241000 ext 20379 e-mail: earanda@ugr.es

Diclofenac (DFC) is one of the most non-steroidal anti-inflammatory compounds (NSAID) used around the world. As a drug, the main mechanism for its pharmaceutical effect is due to an interference of prostaglandin synthesis by the inhibition of the cyclooxygenase enzyme system. Although it has demonstrated its effectiveness as a pharmaceutical compound, in recent years, it has gained environmental importance because it is considered one of the main emerging contaminant (EC) in water bodies. These pollutants are characterized by being persistent in nature, usually at low concentrations, and not having clear legislation about concentration levels in the environment. Previously, we demonstrated the total elimination of DFC in a batch bioreactor by ascomycetes fungi using free and immobilized pellets mycelium (Patented data); here we expanded the analysis of biodegradation of this NSAID by the use of continuous bioreactor based on the batch regime. Defined culture medium with glucose as carbon source and 100 µM of DFC as a final concentration were used in the influent. Pellets of the ascomycetes model were inoculated into the bioreactor that contained the same type of medium. The influent flow was of 2 mL/min and HRT of 24 h. DFC residual concentration was measured by HPLC in the influent, bioreactor and effluent every 6/12 h during 5 days. Also, pH, dissolved oxygen rate, and residual glucose were monitored at all sample points. It was observed that >95% of DFC concentration in the effluent was eliminated in 6 h, reaching its total elimination in the subsequent hours, keeping the same value for the rest of the experiment. Into the bioreactor, there is no accumulation of the pollutant and the total elimination is achieved around 18 h. Other parameters such as pH, temperature, and oxygen, did not show significant changes. Final biomass was 3 times bigger than batch experiments. No glucose residual concentration was found in effluent and bioreactor, suggesting a strong co-metabolic behavior. These results indicate that the use and implementation of batch and continuous bioreactor using ascomycetes fungi for the elimination of the DFC are possible. Additionally, tests under real conditions using wastewater are necessary to continue with the development of this novel technology to implement effluent pollutant removal systems.

Phenylpropanoids associated with the induction of embryogenic response in cultures of avocado (*Persea americana* Mill)

Carol A. Olivares-Garcia, Martín Mata-Rosas, Aldo Segura-Cabrera, Jose M. Elizalde-Contreras, Sandra S. Schettino-Salomón, Nayeli Alonso-Ambrosio, Enrique Ibarra-Laclette, Juan L. Monribot-Villanueva José A.Guerrero-Analco, Carolina Peña-Montes, Eliel Ruiz-May*
Instituto de Ecología A.C
Unidad de Investigación y Desarrollo de Alimentos

*Eliel Ruiz May
Red de Estudios Moleculares Avanzados, Instituto de Ecología A. C., Cluster BioMimic®, Carretera Antigua a Coatepec 351, Congregación el Haya, CP 91070 Xalapa, Veracruz, México. Tel. 228 8421800 ext. 6406
Email: eliel.ruiz@inecol.mx

The establishment of an efficient *in vitro* regeneration system such as somatic embryos in avocado (*Persea americana*) could provide a powerful tool for the mass production of elite plants. However, regeneration of avocado through somatic embryogenesis is difficult due to several negative factors such as poor embryo maturation during transdifferentiation resulting in a low rate of germination. Until now, the molecular foundation of each step of somatic embryogenesis in avocado is a mystery. Furthermore, the bottleneck of embryo maturation could be linked to server metabolic and regulatory pathways before embryo maturation. As the first approach for the global molecular and biochemical characterization of the somatic embryogenesis in avocado we carried out comparative proteomics and metabolomic approach between embryogenic (EC) and non-embryogenic (NEC) cultures generates from immature zygotic embryos of Hass and Criollo avocado cultivars. Our preliminary information suggests that the metabolic pathway of phenylpropanoids is up-regulated in EC. This could be regulated by the overexpression of phenylpropanoid genes during the early stages of embryo development. Besides, key polyphenolic compounds induced the production of more globular embryos compares to control treatment. In this study, we are trying to understand the regulatory mechanism of the induction of more globular embryos which could lead in the long term to resolve the limitation of poor embryo maturation and plant conversion.

Keywords: somatic embryogenesis, proteomics, metabolomics, avocado, phenolic compounds

Understanding the thermal inactivation kinetics of the NADH Oxidase from *Giardia lamblia*

Jesús Oria Hernández*, Adriana Castillo Villanueva,
Sara Teresa Méndez, Horacio Reyes-Vivas.
Laboratorio de Bioquímica-Genética. Instituto Nacional de Pediatría. Secretaria de Salud.
Insurgentes Sur 3700-C, Insurgentes Cuicuilco; Coyoacán, Ciudad de México. *☎(5255) 1084 0900 Ext. 1442 *✉ jesus.oria.inp@gmail.com

In vitro protein stability can be defined as thermodynamic stability or kinetic stability. Thermodynamic stability involves the resistance of a folded protein to denaturation, whereas kinetic stability is related to resistance to irreversible inactivation. For biotechnological processes, kinetic stability is most relevant, since the preservation of the enzyme biological activity is the main goal. Enzyme catalyzed redox reactions have recently gained increasing interest in biocatalysis. We have previously characterized the NADH oxidase from *Giardia lamblia* (GlNOX)[1], a FAD-dependent enzyme that uses NADH or NADPH as electron donors and oxygen as the primary electron acceptor, rendering H_2O as end product without production of $O_2.^-$ or H_2O_2. The capacity of enzymes like GlNOX to oxidize both NADH and NADPH with side products no other than water has been highlighted for its biotechnological potential as versatile and innocuous regeneration systems for redox reactions requiring NAD^+ or $NADP^+$ as cofactor. Since stability can limits the usefulness of an enzyme industrially, in this work we attempt to understand the determinants of inactivation of GlNOX, as a first step to alleviate subsequently its kinetic stability. We study the inactivation of GlNOx at 60°C and the factors modifying the inactivation patterns. Depending on the conditions of the milieu (pH) the inactivation can be monophasic or biphasic, and can be accelerated or retarded. The addition of additives as the coenzyme (FAD) or a binding site ligand (NAD) modifies slightly the inactivation, whereas the addition of DTT has a major protective effect on the inactivation rate. Trehalose, a well-known protein stabilizer, retards the inactivation of GlNOX, but in conjunction with DTT show a synergistic effect that increases substantially the kinetic stability of the enzyme. A model for the heat induced inactivation of GlNOx is presented.

Characterization Of A 3d Construction With Movilized Sheep Bone Marrow Cells Differentiated To Bone And Cartilage

Carmina Ortega Sanchez, Carlos Landa Solis, Zaira Yunuen Garcia Carvajal, Phaedra Suriel Silva Bermudez, David Garciadiego Cazares, Yaaziel Melgarejo Ramirez, Valentin Martinez Lopez, Jose Clemente Ibarra Ponce de Leon.
Instituto Nacional de Rehabilitacion "Luis Guillermo Ibarra Ibarra"
Calzada Mexico Xochimilco 289 Col. Arenal de Guadalupe CP.14389
minaorsa@hotmail.com

Introduction The three-dimensional scaffolds offer great potential to mimic the native extracellular matrix due to its fibrous nanoscale structures. Several fine or avascular tissues, such as skin, cartilage and bladder, have been successfully designed *in vitro.* However, the lack of spatial and temporal control of the 3D cellular microarchitecture and the distribution of the extracellular matrix in three-dimensional scaffolds limits its application. Emerging methods, such as robotic printing, can fabricate three-dimensional complex tissue structures *in vitro* by assembling gels with encapsulated cells. **Objective** To characterize a 3D construct with mobilized stem cells from sheep bone marrow differentiated to bone and cartilage.

Methodology

Two hydrogels were prepared based on alginate at different concentrations, to simulate the components of the extracellular bone matrix β-TCP and PVA cross-linked with CaCl2 were added and for cartilage additional chondroitin sulfate was used, both anchored on a PCL skeleton made by robotized printing, the hydrogels were evaluating microstructure in SEM, porosity and swelling. To evaluate biocompatibility of the polymer, mesenchymal cells were isolated from peripheral blood of a 2-year-old Suffolk sheep. By Ficoll gradients, mononuclear cells were isolated, the cells were labeled with CD34, CD45, CD90, CD105 antibodies where the immunophenotype was established by flow cytometry, the cells were seeded in primary culture until reaching 80% confluence in DMEM at 10% SFB and 1% of antibiotic. Pass 3 was planted on the scaffolds to evaluate viability, proliferation,cytotoxicity and differentiation of the cells towards the chondrogenic and osteogenic lineage.

Results

Two polymers were successfully obtained for the bone and cartilage phases in a satisfactory manner, the phase for chondrogenic cells was performed by encapsulation of cells, the phase for bone is constituted by 58.3% of pores of a range of 80-256µm, no a plateau was observed in the absorption of water after 24 h causing a disintegration of the polymer due to manipulation. For the evaluation of biocompatibility, the mobilized cells of peripheral blood were differentiated and characterized by immunohistochemistry for the chondrogenic lineage resulting positive for collagen II and sox 9 and for the osteogenic lineage positive for osteopontin and RunX2. The polymers were anchored in the base of PCLobserving a viability of 80% The proliferation of the cells stopped when seeding them

on the scaffolding, however, the polymers did not cause cell death.

Conclusions

The formation and characterization of the 3D construct was achieved by observing acceptable physical characteristics; however, properties such as swelling,proliferation and cellular adherence observed indirectly can cause the failure of the constructs at the time of implantation, so it is suggested to continue investigating their functionalization.

Azotobacter vinelandii mutant in *relA/spoT* homologous promotes early degradation of PHB

Cristian Camilo Ortiz Vasco, Ma. Soledad Moreno León and Elda Guadalupe Espín
Instituto de Biotecnología-UNAM
Cod. Postal: 62209 Tel: 3291629. Correo: espin@ibt.unam.mx

The polyhydroxybutyrate (PHB) is a biopolymer that belongs to the family of polyhydroxyalchanoates (PHA). PHB is synthesized by several organisms and it is used as a biodegradable and biocompatible plastic. In *Azotobacter vinelandii* the production/degradation of PHB is highly regulated, its production occurs in stationary phase and depends on availability of carbon. In contrast the mechanism of PHB degradation in *A. vinelandii* is little known. The PHA-depolymerases are necessary for degradation intra and extra cellular. The RelA/SpoT homologous are responsible of the synthesis (RelA/SpoT) and degradation (SpoT) of the secondary messenger (p)ppGpp, which is a master regulator of transcription and translation in bacteria, and it is important in the stringent response caused by the deprivation of aminoacids, phosphates, heat shock, and carbon storage. The importance of (p)ppGpp on different cellular process is currently under study.

In this study, we showed that mutations in genes *avin02810*, avin37060 encoding the RelA and SpoT proteins and double mutant (*relA/spot),* do not have an effect in the synthesis of PHB under the condition tested (PY agar 2% sucrose) relative to the wild type UW136. Interestingly, the degradation of PHB in the mutant *avin37060* and double mutant was enhanced, and the level of PHB was reduced as compared with wild type; Polymer degradation started after four days of incubation at 30°C, while in the wild type the degradation of polymer started after nine days post-incubation. In the single mutant *avin02810* (SpoT) the synthesis or degradation of PHB were similar to the wild type.

In *relA* and *relA/spoT* mutants in PHB producer bacteria, the (p)ppGpp levels are low, have been demonstrated to enhance the activity of PHB depolymerases. In this study, we will determine the mechanism by which (p)ppGpp regulates the degradation of PHB in *A. vinelandii* and the effect on the expression or activity of the depolymerases present in the *A. vinelandii* genome

Untargeted and targeted metabolomics analysis reveals a possible role of flavin monooxygenase YUCCA4 in the production of glucosinolates and plant development in Arabidopsis.

Randy Ortiz-Castro[1*], Juan Luis Monribot-Villanueva[1], José Antonio Guerrero-Analco[1], Aarón Giovani Munguia-Rodríguez[2], José López-Bucio[2].

[1]Instituto de Ecología, A.C., Red de Estudios Moleculares Avanzados, Carretera Antigua a Coatepec 351, El Haya, CP. 91070, Xalapa, Veracruz, México.
[2]Universidad Michoacana de San Nicolás de Hidalgo, Instituto de Investigaciones Químico-Biológicas, Ciudad Universitaria, Edificio B3, CP. 58030, Morelia, Michoacán, México.
*Corresponding author: randy.ortiz@inecol.mx

Plants are sessile organisms exposed to different biotic and abiotic stimuli, which together with the intrinsic genetic program modulate their growth and development through different regulators. The indole-3-acetic acid (IAA) has been described as one of the main regulators of plant development through the modulation of the cell cycle and root architecture, such as primary root growth, lateral root formation and root hairs. Different routes of IAA biosynthesis from tryptophan have been described. One of the routes of biosynthesis of the IAA involves the participation of a flavin monooxygenase enzyme belonging to the YUCCA family of genes that participate in the conversion of indole-3-pyruvate (IPA) to the indole-3-acetic acid, the main auxin in the plants. Previous studies have shown that overexpression of the YUCCA genes induces an overproduction of the IAA in Arabidopsis. Metabolic analyzes have shown an advantage in the understanding of metabolic status, identification of biomarkers and characterization of biological pathways. In the present work, targeted and untargeted metabolomic analysis of wild type and overexpressing Arabidopsis seedlings of the *YUCCA4* gene were performed by using liquid chromatography coupled to accurate mass spectrometry. Our results showed the same levels of IAA production in both the WT and *35S::YUCCA4* Arabidopsis seedlings. Interestingly, we found an increase in the production of several indole glucosinolates as neoglucobrassicin and methoxyglucobrassicin, glucosinolates as 6-(methylsulfonyl) hexyl glucosinolate and flavonol glucosides as kaempferol triglucosides in the *35S::YUCCA4* compared with the WT Arabidopsis seedlings. Previous studies have suggested the possible role of flavin monooxygenases enzymes of the YUCCA family genes in the biosynthesis pathway of indole glucosinolates and our results agreed with their involvement. Also, it has been reported kaempferol as natural inhibitors of polar auxin transport. All data together could be related to the phenotype showed in the overexpressing lines.

SEA CUCUMBERS AS A POTENTIAL MARINE COLLAGEN SOURCE: A HIGH PERFORMANCE METHOD

Jorge A. Osorio-Kuan[1], Alan G. Hernández-Melgar[1], Martha M. Manzano-Mora[1], Miguel Cuevas Cruz[1], Barbarín Arreguín-Lozano[1], Roberto Arreguín-Espinosa[1], Francisco Alonso Solís-Marín[2], Noe Salgado Ortiz[2] and Delia Simental[3]
JAOK: alan.hernandez@mail.com; RAE: arrespin@unam.mx

1 Departamento de Química de Biomacromoléculas, Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Ciudad Universitaria, México 04510, Ciudad de México, México.
[2] Universidad Nacional Autónoma de México, Laboratorio de Sistemática y Ecología de Equinodermos, Instituto de Ciencias del Mar y Limnología, Circuito Exterior S/N, 04510 Mexico City, Mexico
[3] Coco Chavita SPR de RL. Maariano Matamoros 38 Sur, Col. Centro, CP. 82400, Escuinapa, Sinaloa, México.

Collagen is a versatile protein commonly used in biomedical devices as a biomaterial due to its unique properties such as tensile strength, biocompability and absorbability in living tissues (Silva et. al., 2014). The global market size of this molecule has been estimated at $31 billion in 2016 and its expected to growth constantly in the next years (Subhan et. al., 2014). Traditionally, the most commonly used source of this molecule has been porcine and cattle connective tissues. Although the disponibility of these materials is not limited, market restrictions have drawn the attention towards novel sources such as Holothurians (Subhan et. al., 2014). These organisms commonly known as “sea cucumbers” have shown to be a large collagen source, owing to the fact that it accounts 70 % of total protein (Zhong et.al., 2015). In the present work we describe a high performance method for the isolation, purification and structural characterization of the pepsine-soluble collagen, from the body wall of two previously unstudied Mexican holothurian species from the Gulf of California, *Holothuria inornata* and *Holothuria lubrica.*

Key words: *Holothuria inornata*, *Holothuria lubrica*, Purification method, Pepsin-soluble collagen.

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The use of a radio-sterilized fascia lata as a scaffold of the mesenchymal stem cells derived from the adult Hoffa´s fat package

Biol. Ma. Dolores Ozuna Arroyo. Biol. José Dante Daniel Gómez Cuautle. Dr. José Clemente Ibarra Ponce De León. Dra. María *Cristina* Velasquillo Martínez. Biol. Carmina Ortega Sánchez. M.C. Félix Enrique Villalobos Córdoba. Biol. Alberto Carlos Martínez. Dr. Daniel Luna Zaragoza. M. en C. Valentín Martínez López.

Instituto Nacional de Rehabilitación
M en C Valentín Martínez López.
Tel: (+52) (55) 59 99 10 00 Ext. 19604 y 19610.
email: val_mart76@yahoo.com.mx

At soft tissue level the most typical lesions occur in tendons and ligaments, where between 10 and 40% are presented in the rotator cuff. Several complications such as re-ruptures and production of nonfunctional scars happen with conventional treatments for this type of injuries. The superior capsular reconstruction where a radio-sterilized fasciae lata autograft is used as the augmentation material due to the pro-inflammatory immune response can be a treatment option for irreparable rotator cuff tear. Therefore tissue engineering and regenerative medicine have developed treatments for this type of injuries using scaffolds which are materials that mimics the tissue to be regenerated, these can be complemented with the use of mesenchymal stem cells (MSCs) and active biological signals to promote differentiation, proliferation and cell growth. Hence the use of MSCs could improve therapies of regeneration and repair of the rotator cuff due to its high plasticity rate, self-renewing capacity and immunomodulators. The objective of this work was to demonstrate that the fascia lata could be seen like a scaffold for the MSCs derived of Hoffa adipose package (Ad-hMSC); the immunophenotype, proliferation time and plasticity were evaluated. Five samples of Ad-hMSC from donors with inform consent were characterized. Our results showed that the Ad-hMSC present the immunophenotype positive for: CD 90, CD 105, CD 44, CD 73 and CD 166 and negative for immunophenotype of hMSC: HLA-DR, CD 34 and CD45 established by the International Society of Cellular Therapy; Ad-hMSC had a doubling time of 42 h, which are UFC. They showed differentiation capacity to adipogenic, chondrogenic and osteogenic linage. The fascia lata decellularized showed <90% DNA content. Viability of the Ad-hMCS was evaluated on the radio-sterilized fascia lata and decellularized for 15 days in vitro. With electronic microscopy we observed that Ad-hMSs produce extracellular matrix and fibroblastic like phenotype as tenocyte cells. With this study it has been verified that the fascia lata can be used as a scaffold for Ad-hMSC due to its biocompatibility, making it a potential graft for the use in therapy of rotator cuff injuries helping to diminish immune responses.

Functional characterization of two patatin-related phospholipases A (pPLA) of *Phaseolus vulgaris* during nodulation

Ronal Pacheco[1], Marco Juárez-Verdayes[1], Jorge Solís-Miranda[1], Noreide Nava[1], Yolanda Ortega-Ortega[2], Citlali Fonseca[1], Alfonso Leija[3] and Carmen Quinto[1*]

[1]Departamento de Biología Molecular de Plantas, Instituto de Biotecnología. Universidad Nacional Autónoma de México. Av. Universidad # 2001, Col. Chamilpa, C. P. 62210, Cuernavaca Morelos, México.
*quinto@ibt.unam.mx
[2]Centro de Investigación en Química Aplicada, Conacyt. Blvd. Enrique Reyna, # 104, C. P. 25294, Saltillo, Coahuila, México.
[3]Programa Genómica Funcional de Eucariotes. Centro de Ciencias Genómicas. Universidad Nacional Autónoma de México. Av. Universidad, Col. Chamilpa, C. P. 62210, Cuernavaca Morelos, México.

Phospholipases A2 are hydrolytic enzymes of phospholipids, that in plants are classified according to their protein sequences and biological characteristics. Some of them are identified as patatin-related phospholipases A (pPLA) since they have a patatin domain. These enzymes are involved in response to pathogens, vegetative grow and cellular elongation. However, the participation of pPLA in mutualistic plant-microbe interactions has been poorly explored. Previously, a transcriptomic analysis of *Phaseolus vulgaris* roots with a loss or gain-of-function of a NADPH oxidase gene, was obtained by RNA-seq; the total RNA of these transgenic roots were obtained while in symbiosis with the rhizobacteria *Rhizobium tropici* or with *Rhizophagus irregularis,* an arbuscular mycorrhizal fungi. Two genes (*Pvflap-IIα* and *β*) were identified with a presumed antagonistic effect in both types of symbiosis. These genes have amino-acid sequences strongly related to pPLA. Nineteen pPLA were identified, whose amino-acid sequences have highly conserved regions with pPLA of other species. According to their phylogenetic relationships, these proteins were located into three clades. Quantitative analysis of transcript levels of *Pvflap-IIα* and *β* in bean roots non-inoculated or inoculated with *R. tropici*, showed that *Pvflap-IIα* present higher transcript accumulation when compared to *Pvflap-IIβ*. Transcript levels of *Pvflap-IIα* did not show significant differences between bacterial inoculated and non-inoculated roots within the first seven days after inoculation. In order to reveal the participation of *Pvflap-IIα* on rhizobial symbiosis, specific down-regulation or over-expresion of this gene was achived. Currently, phenotypical characterization of the generated transgenic common bean roots with loss or gain-of-function of *Pvflap-Iiα is in progress.*

Key words: nodulation, patatins, *Phaseolus vulgaris,* phospholipases

Biochemical Characterization of Wild Yeasts Producing Ethanol

[a] Román Bruno Paz García, Álvarez Quiroz Ernesto, *Fuentes Ramírez Luis Ernesto, *Castañeda Antonio Dolores, * Avelino Flores Fabiola y *Santoyo Páez Yuriria.
Facultad de Ciencias Biológicas, Benemérita Universidad Autónoma de Puebla.
Licenciatura de Biotecnología, Benemérita Universidad Autónoma de Puebla.
*Centro de Investigación en Ciencias Microbiológicas-ICUAP, BUAP, Puebla, Correo: brun002295@gmail.com, cel.: 2225212464, C.P.: 72589

Introduction: Yeasts play a very important role in fermentation industry, to produce beverages, food and other products such as fuels, as well as to be used as model in genetics tests, recombinant proteins production, etc. The strains of the genus *Saccharomyces* are the most used worldwide to perform fermentative processes due to their ideal characteristics such as tolerance to ethanol and low pHs, flocculation, genetic stability and their ability to assimilate a range of carbohydrates. Isolation and characterization studies have been carried out throughout the world looking for yeasts which may provide unique characteristics in fermentation, for example in India and England (Tikka et al., 2013) isolates from fruits, grains and molasses have been reported, in addition to the beer production evaluation (Lentz et al., 2014); as well as in breweries in the USA where wild yeasts are isolated and characterized to produce new flavors (Delong et al., 2017). In Mexico isolations of yeasts of the genus *Saccharomyces* have also been carried out mainly in vineyards and distilleries (Miranda-Castilleja et al., 2015), however, there are not many studies directed to the search of wild yeasts for the brewing industry or they have not been reported. This study will allow obtaining yeasts with a suitable profile for the production of craft beer.

Objectives: Characterize and evaluate wild yeasts for the production of ethanol.

Methodology: Yeasts were isolated from lignicolus mushrooms, plum, pineapple and Mexican *pulque*, in selective, low-cost media; they were characterized by their colonial and microscopic morphology at a 100x magnification; after that, biochemical tests were carried out: urease test, assimilation and fermentation of sugars to approach the genus *Sacchcaromyces* and reduce the number of yeasts to be evaluated. Finally, tolerance to ethanol, pHs and temperature tests were carried out.

Results: -The isolation method was standardized and it was possible to is
olate 106 wild yeasts, from which, only 21 strains (19% of the yeasts) assimilate and ferment glucose, sucrose and maltose, only assimilate raffinose and do not assimilate mannitol, sorbose, xylose, lactose and arabinose, besides, they do not assimilate urea; this indicates these yeasts show a biochemical profile probably of the genus *Saccharomyces cerevisiae*. In addition 7 of these yeasts support a 10% volume of alcohol content in the medium.

Conclusions: These 7 yeasts may belong to the genus *Saccharomyces cerevisiae* and they present characteristics suitable for ethanol production.

Topic Area: Biotechnology and Fundamental Biochemistry.

In vitro production of Huperzine A by cell suspension cultures of the plant species *Huperzia orizabae*

Rocio Pérez[1], Talia Rodríguez[1], Olga Cruz[1], María Luisa Villarreal[1], Anabel Ortiz[1], Alexandre Cardoso-Taketa[1].
[1] Centro de Investigación en Biotecnología. Laboratorio de Biotecnología en Plantas Medicinales.Universidad Autónoma del Estado de Morelos.
Av. Universidad 1001. Colonia Chamilpa, C.P. 62209, Cuernavaca, Morelos. email: rociobio2103@gmail.com

Alzheimer's disease (AD) is considered to be a progressive and irreversible neurodegenerative disorder, characterized by memory loss and damage to cognitive functions, both related to neuronal death1. Huperzine A (HupA) is a selective reversible inhibitor of the enzyme acetylcholinesterase2, which has attracted generalized attention due to its unique pharmacological activity and its low toxicity to treat AD. In recent years, plants belonging to the Huperziaceae family have been studied as an alternative source for the production of HupA, as is the case of *Huperzia orizabae*3, an endemic species of Mexico. Due to the importance of HupA in the treatment of AD, there has been an excessive harvesting of species such as *H. serrata,* the first fern exploited from its natural habitat, for HupA production, which puts it in danger of extinction. The present research focuses on the development of biotechnological strategies to establish a profitable production of HupA using cell suspension cultures of *H. orizabae* obtained from calluses previously generated in our lab.

For the establishment of the suspension cultures, calluses of *H. orizabae* were cultured in baffled flasks with B5 medium added with the phytoregulators 1-Naphtalenacetic acid and Zeatine, both at of 0.5 mg/L, in constant light, agitation at 100 rpm and 25 ° C. A kinetic study was carried out during 60 days with sampling each 5 days. Parameters as mass growth (fresh and dry weights), the presence of HupA, pH, and cell viability, were measured. Extraction of alkaloids of the dry biomass was carried out for HPLC quantification.

After 45 days in culture, the growth kinetics indicated that the biomass increased 4.18 times, obtaining 8.13 g dry weight (DW) with respect to the initial inoculum (1.94 g DW). The production of HupA during the time in culture varied between 0.42 µg/g DW (day 0) to reach a maximum production of 16.52 µg /g DW (day 45). The stationary phase started at day 35. This investigation constitutes one the first report on the establishment of *in vitro* cell suspension cultures of species of the Huperziaceae family3.

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Electrochemical analysis of the redox metabolites produced by *Pseudomonas aeruginosa* NEJ01R.

Pérez-Garcia, Jesús Alberto; Bácame-Valenzuela, Francisco Javier; Ortiz-Frade, Luis; Reyes-Vidal, Y.
Centro de Investigación y Desarrollo Tecnológico en Electroquímica, S.C.
Parque Tecnológico Querétaro s/n, Sanfandila, Pedro Escobedo, Querétaro. C.P. 76703, México.
jgarcía@cideteq.mx; mreyes@cideteq.mx

Pseudomonas aeruginosa NEJ01R is a strain isolated from the effluents of nixtamalization, a process for obtain tortilla. Some species of genus *Pseudomonas* produces pyocyanin, an extracellular metabolite[1]. This metabolite is a reversible redox molecule, with an important role in communication cell to cell and bacterial cellular respiration[2]. An electrochemical analysis of the molecule can help to understand this process through its redox activity. The objective of this work was to study by cyclic voltammetry the metabolites present in the supernatant of a liquid culture of *Pseudomonas aeruginosa* NEJ01R. Microorganism was cultured in Luria Bertani liquid medium, incubated for 72 h, at 76 rpm and 30°C. The supernatant was obtained by centrifugation and extraction with chloroform. An analysis was performed by chromatography UPLC coupled to Photo Diode Array Detector for analyze compounds in supernatant. The electrochemical studies were carried out in a three-electrode cell, phosphate buffer 0.1 M at pH = 7 and a potentiostat/galvanostat. Chromatographic analysis indicated the presence of different molecules, including pyocyanin. The voltamperometric analysis in oxidation mode indicated the presence of two reduction processes, Ic and IIc, with values of Epc (I) = -0.203 V vs REF and Epc (II) = -0.319 V vs REF. The inverse process showed two peaks, Ia and IIa, related with oxidation processes. The values obtained were Epa (I)= -0.177 V vs REF and Epa (II) = -0.235 V vs REF. Thus, the strain of *Pseudomonas aeruginosa NEJ01R* produces biomolecules with electrochemical reversibility. The culture conditions suggest that the production of pyocyanin and other redox compounds are related to the low concentration of dissolved oxygen, according to the low agitation and because their signals are manifested at cathodic potentials close to the formation of the superoxide ion. An analysis comparative of the data obtained in this work will be presented with the characterization of redox substances produced by other *Pseudomonas* species[3-5].

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BIODEGRADATION OF BISPHENOL A BY *Pleurotus ostreatus* GROWN IN SUBMERGED FERMENTATION AND EFFECT ON LACCASE ENZYMES ACTIVITY

[1]Georgina Pérez-Montiel, Jorge Alberto Rubio-Piña, Libertad Juárez-Santacruz, Diana Verónica Cortés-Espinosa, José Luis Torres-García, Gabriela Córdoba-Sosa y [1]Miriam Ahuactzin-Pérez.
[1]Autonomous University of Tlaxcala, Faculty of Agrobiology, Degree in Biology, Food Production and Processing Laboratory. Ixtacuixtla, Tlaxcala.
E-mail: miriamahuactzin@gmail.com
georginaperezmontiel@gmail.com
Phone: 2461119749

Bisphenol A (BPA) or 2,2-bis- (4-hydroxyphenyl) propane is an organic compound, used as a plasticizer. Their entry into the environment occurs in different ways. This can be degraded by microorganisms: fungi such as *Pleurotus ostreatus*. Different parameters of the fungus were evaluated in concentrations of 50 and 75 mg/L of BPA, specific growth rate (μ), maximum biomass produced (Xmax), glucose consumption. Percentage and constant biodegradation (%*k) of the BPA, enzymatic activity of laccases (U/L), yields of the enzyme with respect to the substrate ($Y_{E/X}$), enzymatic productivity ($P=E_{max/h}$), maximum enzymatic activity (E_{max}), specific rate of formation of the enzyme $qp=(\mu)*(Y_{E/X})$.

The yields obtained with respect to μ, X_{max} and E_{max} were higher in the medium of higher concentration. *P. ostreatus* showed acid pH values at 0 concentration increasing for the 50 and 75 mg/L BPA media. The highest activity of laccases was 219 U/L produced during the 312 h of growth in the medium of higher concentration. *P. ostreatus* showed a good efficiency in biodegradation of BPA obtaining more than 50% of the degradation during the first 72 h of growth, one k of 0.0051 and 0.0045 h^{-1} in the media of 50 and 75 mg/L of BPA, respectively. These results suggest that *P. ostreatus* uses BPA as a secondary source of carbon and that it shows a positive effect on the production of laccases, and can be used for bioremediation processes of sites contaminated by this type of compounds.

Effect of the extracts of the plants Bouvardia ternifolia and *Solanum nigrescens* on the growth of *Escherichia coli*

José Luis Torres-García, Gabriela Córdoba-Sosa, Binicio Ramírez-Mendoza, Alberto de Jesús Ortíz-Zamora, Georgina Pérez-Montiel, Miriam Ahuactzin-Pérez

Laboratorio de Biología Experimental, Facultad de Agrobiología, Universidad Autónoma de Tlaxcala, Autopista Km 10.5, Ixtacuixtla de Mariano Matamoros, Tlax., C.P. 90120, biolfungus@gmail.com

The great diversity of plants and the rich cultural wealth of Mexico have favored the use of plants for medicinal purposes, thanks to its multiple bioactive components, which produce various curative effects against diseases that cause pathogenic microorganisms. The objective of the present work was to determine the antimicrobial activity of ethanolic and methanolic extracts obtained from *Bouvardia ternifolia* and *Solanum nigrescens* on the growth of *Escherichia coli*. To obtain the extracts, 10 g of each plant dehydrated in 250 mL of ethanol and pure methanol were used. The extracts were named according to the following nomenclature: for the plant *B. ternifolia* with ethanol (BEE) and with methanol (BME), for the plant *S. nigrescens* with ethanol (SEE) and with methanol (SME). Inhibitory concentration 50 (IC^{50}) was measured using different concentrations of each extract. 1.8 x 10^{-4} UFC of *E. coli* was used as inoculum. The survival % and the mortality % of *E. coli* were obtained. The IC50 value was obtained by plotting the Ln of the [ ] of each treatment vs the% mortality of *E. coli*. In the same way, the survival constants (*Ks*) and the mortality constant (*Km*) were obtained. The antimicrobial activity was evaluated by the disc diffusion technique. The highest IC50 was shown in the SEE, however, the highest mortality %and the highest antimicrobial activity was shown in the BME. The best survival percentage of *E. coli* in the extracts was shown in BME. The highest percentage of *E. c*oli mortality was shown in the BME followed by BSE. The highest antimicrobial activity was shown in the BME and SME. The results obtained from% mortality and the IC^{50} of the *B. ternifolia* plant showed greater antimicrobial action on *E. coli*. *B. ternifolia* could be a plant used in the pharmaceutical industry as a model for the generation of new antibiotics with greater spectrum of action.

ENTOMOCHEMICALS PRESENT IN DIFFERENT PHENOLOGICAL STATES OF THE CENTRAL AMERICAN LOCUST (*Schistocerca piceifrons piceifrons,* Walker)

Rogelio Pérez-Ramírez[1], Ludivina Barrientos-Lozano[1] y Jorge Ariel Torres-Castillo[2]

[1]Tecnológico Nacional de México-Instituto Tecnológico de Ciudad Victoria-División de Estudios de Posgrado e Investigación. Blvd. Emilio Portes Gil No. 1301. C.P. 87010. Cd. Victoria, Tamaulipas, México.2Universidad Autónoma Tamaulipas-Instituto de Ecología Aplicada. División del Golfo No. 356. C.P. 87019. Ciudad Victoria, Tamaulipas.
roge_pe_ram@live.com.mx, ludivinab@yahoo.com, joatorres@docentes.uat.edu.mx

Animals, like plants and microorganisms, produce specialized compounds called secondary metabolites, which have implications in all ecological interactions and also in regulation of physiological processes. In the case of insects, it has been observed that these compounds are widely diverse; many of them are synthesized by the organism itself, while others are accumulated from their diet. Some of these compounds have been highlighted because of their potential for biotechnological applications. Insects are considered an important group to be explored as a source of bioactive compounds. Orthoptera is an interesting group because of their diversity, size, great variety in their diet and high reproductive rate. This work aimed to determine the secondary metabolites in different phonological stages of the Central American locust (*Schistocerca piceifrons piceifrons*, Walker) (Orthoptera: Acrididae). *S.p. piceifrons* is an economically important pest for Mexican agriculture, particularly in southern Tamaulipas. This pest presents generalist feeding habits and causes severe damage to several crops. Its nymphal stage passes through six instars that vary in color and preference in their diet. They constitute an interesting study model to explore the presence and variation of entomochemicals (synthesized or accumulated from diet). These entomochemicals or secondary metabolites could have potential for biotechnological applications. As a result of the initial exploration of *S.p. piceifrons*, using colorimetric and spectrophotometric tests, the presence of phenolic compounds, alkaloids, sponins, flavonoids and antioxidants against the DPPH and ABTS radicals has been determined. The detection of digestive enzymes whit trypsin like proteolytic activity as a mechanistic type reported in several orders of insects has also been detected. These allow us to consider this species as a source of bioactive compounds of biotechnological interest, mainly for pharmaceutical or food components.

Partial purification of an intracellular β-glucosidase from *Clavispora lusitaniae*

Odilia Pérez-Avalos, David Ramírez Salmerón, Erika Alfayuset Ochoa & Teresa Ponce Noyola. Department of Biotechnology and Bioengineering. Cinvestav-IPN; Av. IPN 2508, Col. San Pedro Zacatenco México, D.F. CP. 07360; tponce@cinvestav.mx
Key words: β-*glucosidase, cellobiose and glucose.*

Introduction

The β-glucosidase (β-D-glucoside, glucohydrolase, EC 3.2.1.21) is a key member of cellulase enzyme complex that hydrolyzes glycosidic bonds (β-1,4) between two glucose molecules (cellobiose) and other β-linked oligosaccharides with release of glucose (1). The cellobiose is a disaccharide that accumulates during saccharification of lignocellulosic material becoming a bottleneck during the production of bioethanol, because it inhibits the activity of endo and exoglucanases. There are extracellular (2) and intracellular β-glucosidase produced by microorganisms (3). In this work was studied an intracellular β-glucosidase from *Clavispora lusitaniae.*

Materials and methods

Induction and production

C. lusitaniae was grown in a 125 mL shake flask with 25 mL of Breus minimum medium with 10 g/L cellobiose (Sigma) as carbon source and inducer. The culture was incubated at 30 oC, 150 rpm for 12 h.

Purification

Cells were harvested and lysed using glass beads (2 mm diameter) and vortex for 30 min. The homogenate was centrifuged (14, 000 rpm, 4oC, 45 min), supernatant was recuperated. The supernatant was passed through to three purification columns: QSepharose, molecular exclusion BioGel P-60 and Sephacryl S-200, respectively. In all cases, 25 mM Tris-HCl (pH 7.2) was used as a binding and elution buffer. Fractions with β-glucosidase activity were pooled, concentrated and analyzed by SDS-PAGE. Molecular mass of the partially purified enzyme was estimated by SDS-PAGE (10%) (4). Enzyme activity assays were measured as previously reported (5).

Results and discussion

C. lusitaniae produced an intracellular β-glucosidase, that was induced 30 times by cellobiose compared to glucose. The β-glucosidase was partially purified enhanced almost 7 times its specific activity (Table 1) after the different purification steps. Its molecular mass, estimated by SDS-PAGE, was approximately 90-94 kDa (Figure 1A). The β-glucosidase from *C. lusitaniae* presented activity using 5 mM MUG as substrate (Figure 2B).

Table 1. Protein purification of β-glucosidase from *C. lusitaniae*

Purification step	Specific activity (U/mg)	Total Activity (U)	Purification fold
Crude cell extract	474.3	20315.7	1.0
Q-Sepharose	576.4	5509.4	1.2
Bio Gel P-60	706.2	5231.5	1.5
Sephacryl S-200	3257.3	2627.5	6.9

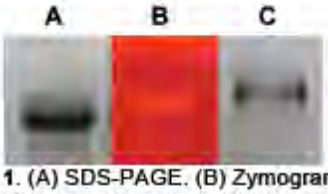

Figure 1. (A) SDS-PAGE. (B) Zymogram with 5 mM MUG as substrate. (C) Native PAGE.

Conclusion

An intracellular β-glucosidase of *C. lusitaniae,* with a molecular mass between 90-94 kDa, was purified.

Acknowledgements

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Physiological role of cytokinins in *Trichoderma atroviride*

María Daniela Porras Troncoso[1], Vianey Olmedo Monfil2, Alfredo Herrera Estrella[1*]. **1**Laboratorio Nacional de Genómica para la Biodiversidad. Centro de Investigación y de Estudios Avanzados del IPN (CINVESTAV-IPN). Libramiento Norte Carretera León Km 9.6, C.P, 36824, Irapuato, Gto., México. **2**Departamento de Biología. DCNE. Universidad de Guanajuato. Noria Alta SN C. P. 36050, Guanajuato, Gto., México. (462) 166 3000 ext. 3070 alfredo.herrera@cinvestav.mx

Cytokinins (CKs) are phytohormones with an adenine nucleus and an aromatic or isoprene-derived side chain at the N6 position. CKs participate in plant development and physiology. In plants the synthesis and signaling pathways of CKs involves the activity of different genes. In *Arabidopsis*, the biosynthesis of CKs is controlled by the *ipt* gene family, two cytochromes P450 and the Lonely Guy gene family (*log*). An alternative route involves a tRNA-IPT. The CKs signaling pathway triggers a phosphorylation cascade, which begins when the hormone binds to the extracellular domain of its specific receptor (AHKs). Subsequently, the signal is transduced by the phosphorylation of histidine phosphotransferase proteins (AHP) and different classes of response regulators (ARR).
Organisms other than plants, such a fungi, also produce CKs. The *ipt* and *log* orthologous genes are present in their genomes, suggesting that CKs could play a role in several physiological processes, as has already been reported for some phytopathogens, where CKs are involved in hyphae development, nutrient absorption and virulence. *Ipt* and *log* gene orthologs are also present in *Trichoderma*'s genomes, which as a beneficial plant endophyte and an antagonistic agent of other fungi. This suggests that CKs could participate in some physiological process in fungi, other than pathogenicity, which is what has been studies so far.
In this work we will analyze if *T. atroviride* is able to produce CKs as well as if it contains the functional elements for the perception and transduction of the signal triggered by CKs. The results that we obtain will allow us to determine the role of the CKs in the own physiology of *Trichoderma*, in its antagonistic activity against fungi and in the beneficial association that it establishes with plants.
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Shrimp waste used for biotechnological purposes

Laura Itzel Quintas Granados, Lizbeth Ileana Fuentes Torres, Paula Anette Landa Ramírez
Universidad Mexiquense del Bicentenario Unidad de Estudios Superiores Tultitlán. Av. Ex Hacienda de Portales S/N, Col. Villa Esmeralda, Tultitlan, México. C.P. 54910. Tel. 5530234593.
laura.quintas@umb.mx

Polymers has an important role in medial, pharmaceutical and biotechnological industries. The constructions, electronics and biotechnological industries demand high performance polymers for special environments or low-cost polymers. Most of the polymers are produced by chemical methods. However, majority of them are not biodegradable, so its synthesis and final disposable might contribute to environmental problems. In these way, biopolymers offer several advantages in comparison to chemical polymers, such as their excellent physical, chemical and mechanical properties, biodegradability among others. The global market size of biopolymers was USD 2.66 Billion in 2015 and is projected to reach USD 5.08 Billion by 2021. One of the most abundant biopolymers is chitin, poly (β-(1-4)-N-acetyl-D-glucosamine) which is a natural polysaccharide of major importance, first identified in 1884. This biopolymer is synthesized by an enormous number of living organisms; and it is considered the most abundant polymer after cellulose due the amount of chitin produced annually in the world. In nature, chitin exists as ordered crystalline microfibrils forming structural components in the exoskeleton of arthropods or in the cell walls of fungi and yeast. Our objective is isolate chitin from marine crustaceans wastes by chemical methods to obtain chitin and characterize this biopolymer for further biotechnological purposes. The main sources of chitin used in this project were marine crustaceans wastes such as shrimp and crabs. Shrimp wastes were drying using heat air, following by a decolorization treatment to remove pigments to obtain a colorless product. The chitin was extracted by two major steps. First, dry shrimp wastes were subjected to an acidic treatment to dissolve calcium carbonate. Then, we used alkaline extraction to solubilize proteins to finally obtain chitin. The resulting chitin was characterizing in terms of purity and color. Further studies need to be performance to determinate residual protein and pigment contaminations that might cause problems to use the resulting chitin in several biotechnological applications.

Regulated plasmid construction to express a bioactive lectin of *Phaseolus acutifolius* in *Trichoderma reesei.*

Ramírez-Baltazar S. Esquivel-Naranjo E.U. Blanco-Labra A. Gasca-García T. Cervantes-Chávez J.A. Landeros-Jaime F.
Unidad de Microbilogía Básica Aplicada, Campus Aeropuerto, Facultad de Ciencias Naturales de la Universidad Autónoma de Querétero, Anillo Vial Fray Junípero Serra Km 8 S/N, Querétaro Qro. C.P. 76140. ulises_esquivel2000@yahoo.com.mx

Abstrac.
The lectins are glycoprotein, with at least one carbohydrate recognition domain. The binding of protein to carbohydrates does not imply any enzymatic modification or cleavage of these carbohydrates. The lectins have been detected in plants of the families like *Leguminoseae* nad in plants, lectins are distributed in various tissues. Many plants contain lectins, such as wheat, rice, soy and beans. In particular the phytohemagglutinins of *Phaseolus* gender have the ability to inhibit some types of cancer, particulary the lectin of *Phaseolus vulgaris* has been indicated to inhibit the proliferation of different lineages of cancer cells, these lectins have a great potential in the treatment of this type of diseases, it is known that lectins are responsible for cytotoxic effects and that they have a differential anti-proliferative effect on untransformed cells, that is why molecular tools have been developed that allow the expression of these proteins. In yeasts the heterologous expression of proteins is sometimes affected due to differences in the glycosylation pattern of the enzyme. The filamentous fungi *Trichoderma reesei* have a high capacity to secrete large amounts of proteins in the growth medium has the ability to produce heterologous proteins and as a further advantage, the production of the protein of interest can be regulated by factors such as, carbon source or light. It is important to generate adjustable plasmids, which can be induced, since this allows great control over the system and avoid possible adverse effects such as mistake in glycosylation or protein folding due to the metabolic load, commonly induced, in those on constitutive expressions or of the interaction of the recombinant protein in the host cell. It has been considered as a good system for the expression of proteins, since they can also express the structure of the original protein and the correct post-translational modification, this is important in particular for those proteins in which the activity depends on its molecular structure, for example glycosylation and methylation, among others, which are necessary for the correct folding and functioning of proteins. The systems of expression of heterologous proteins in the filamentous fungi in liquid culture, have a great capacity to produce proteins, being an option to generate higher yields of recombinant protein at low costs, however, it is important to generate plasmids that can be regulated and that be able to induced , this allows a greater control over the system and avoids possible adverse effects caused by the metabolic load, it is also essential to generate a system that does not compromise the biological activity and obtain higher percentages of protein recovery.

IN SILICO ANALYSIS OF A METAGENOMIC OBTAINED FROM MARINE SPONGES: A LIGNOCELLULOLYTIC PROSPECTION

Daniela D. Ramírez-Escamilla[1], Heidy Peidro-Guzmán[1], Deborah González-Abradelo[1], Sonia Dávila-Ramos[1], Jorge Luis Folch-Mallol[1], Steve Jackson[2], Alan Dobson[2], Ramón A. Batista-García[1].

[1]Universidad Autónoma del Estado de Morelos, México.
[2]University College Cork, Irlanda.
Centro de Investigación en Dinámica Celular UAEM. Av. Universidad 1001, Col. Chamilpa, C.P. 62209. Cuernavaca, Morelos. México. Tel: +52-777-3297000 ext. 3524, 4550, 3276

In recent years, metagenomics has become in a powerful tool to identify new enzymes with biotechnological value. Functional metagenomics allows the analysis of the genetic reservoirs of those non-culturable organisms and their metabolic prospecting. The search for lignocellulolytic enzymes is an international priority due to its use in industries such as biorefineries. Most of the international energy consumption comes from fossil fuels such as oil, and, only a 20% is derived from renewable sources. Biomass is the most abundant natural resource in the world, but at the same time, it is underexploited for energy purposes. Biomass is mainly composed of cellulose, hemicellulose, pectin and lignin. Lignocellulose is considered a polymer highly resistant to microbial degradation. In this work, a metagenome associated with the marine sponge *Stelleta normani* isolated from Irish deep-sea (750m) was explored. Twenty-five clones with the ability to degrade xylan and cellulose were identified through functional bioprospecting assays. Metagenomic inserts were sequenced and later assembled. Finally, the ORFs (open reading frames) were identified and annotated. The genes coding for cellulases, xylanases, laccases and peroxidases were identified, finding a total of 165 genes. Once the sequences are obtained, their primary and tertiary structure will be analyzed, as well as the phylogenetic relationships with other enzymes of the same family previously characterized. The substrate binding motifs and the amino acids involved in the catalytic site will also be identified to access the most novel variants of the enzymes. The work concludes with the selection of some of these enzymes as candidates to be cloned, expressed and biochemically characterized.

Characterization of Cell Wall Resident Proteins NCW-3, ACW-1 and CCG-6 as Possible Anchors for Protein Display in *Neurospora crassa*

Ana Sofía Ramírez-Pelayo and Jorge Verdín
Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, Unidad Zapopan (CIATEJ), Camino Arenero #1227, Zapopan, Jalisco, México, 45019. (33) 33455200 ext. 2103, jverdin@ciatej.mx

Cell wall protein display is a technology that can endow a microorganism with new functionalities or enhance its existing ones by anchoring an enzyme or other protein to a cell wall resident protein (CWP). Most of the work done in this field has been performed in yeasts, with a minor fraction in bacteria and phages, but almost nothing in filamentous fungi. *Neurospora crassa* is a filamentous fungus which shows promising perspectives in terms of biotechnological applications due to its life cycle, simple culture requirements and innocuousness. In the present study, the functional compromise of three cell wall proteins of *N. crassa* were characterized to assess their suitability as molecular anchors for the display of proteins on the cell wall of this organism: NCW-3 (non-anchored cell wall protein 3, NCU07817), ACW-1 (anchored-cell wall protein 1, NCU08936) and CCG-6 (clock-controlled protein 6, NCU01418). These proteins are representative of different chemical interactions between the cell wall and CWPs and so the best anchor for a specific enzyme can be determined in a way that the incorporation pathway of the CWP into the cell wall does not interfere with the catalytic and structural features of the displayed protein and vice versa. Vegetative growth rate, hyphae morphology and sensitivity to Calcofluor-white (CFW) were measured for the knock-out (KO) strains of each protein and compared to those of the wild type (WT). The KO strains simulate the extreme scenario in which the display fusions cause a deleterious effect on the CWPs and, then, the feasibility of the fusion can be assessed. *Δacw-1* behaved similarly to WT in terms of growth rate (2.43±0.21 mm/h vs 2.60±0.19 mm/h) and sensitivity to 0.45 mg/mL CFW (1.52±0.04 mm/h vs 1.46±0.07 mm/h) which points to the idea that it is not essential to keep the cell wall structure. Interestingly, this mutant displayed tricorned hyphae. *Δncw-3* had a slower growth rate than WT and *Δacw-1* (0.71±0.19 mm/h), but it was the less sensitive KO strain to 0.45 mg/mL CFW (inhibition of growth of 79% vs 76%, 81% and 100% for WT, *Δacw-1* and *Δccg-6*, respectively), which indicates that NCW-3 has an important structural role in the cell wall but its absence does not result lethal. *Δccg-6* had the slowest growth rate (0.44±0.06 mm/h) of all the strains and was the most sensitive to 0.45 mg/mL CFW (100% inhibition of growth), which suggests a vital role in the cell wall integrity. This crucial role is supported by the high-degree of sequence conservation across all the screened homologs of CCG-6 described by a bioinformatic analysis. ACW-1 may be the most suitable anchor candidate for display of proteins because its deletion does not seem to affect the cell wall; in addition, its tricorned tip offers an opportunity to study its role in hyphae polarized growth. NCW-3 is the alternative option. While CCG-6 might not be a suitable anchor since it is crucial to cell wall integrity, it deserves an insightful analysis for its role in cell wall structuration. This work was supported by a grant from SENER-CONACYT “Sustentibilidad Energética” (245750).

Fungal-biosynthesized silver nanoparticles (AgNPs) and its use to prevent biofilm formation of Uropathogenic *Escherichia coli* (UPEC).

Rosa María Ramírez Santoyo, Candelario Rodríguez Serrano, Jesús Guzmán Moreno, Vicente Rodríguez González, José Juan Ortega Sigala, Carlos Ángeles Chavez, Sergio Hugo Sánchez Rodríguez y Luz Elena Vidales Rodríguez**§.**
Unidad Académica de Ciencias Biológicas, Universidad Autónoma de Zacatecas (UAZ).
§Preparatoria s/n, Col. Agronómica II, CP 98066. Zacatecas, Zac. México. 01 (492) 869 05 44.
luzelenavr@uaz.edu.mx

The mining activity in the Zacatecas state in Mexico produces a lot of waste that is deposited on the soil's surface, this produce serious disturbances to the environment, however, the study of filamentous fungi adapted to the presence of highly toxic concentrations of metals in soil, as well as the metal-microbe interactions, provides an opportunity for the establishment and characterization of fungal processes involved in the metal nanoparticles (NPs) biosynthesis with applications in the treatment of cancer, biosensor design, catalysis, optical receptors, targeted drug delivery and antibacterial uses. This study shows the identification and characterization of the Ag-NPs produced of a silver-tolerant fungi strain isolated from a metal-contaminated soil of Zacatecas and the evaluation of the antibacterial effect of the biogenic AgNPs over both planktonic cells and biofilms of the human Uropathogenic *Escherichia coli* (UPEC). The morphological analysis and molecular identification by sequencing of ITSs from rRNAs genes, determined that the fungal isolate Ag5-0.5 belongs to *Fusarium equiseti* specie. The capacity of this fungal strain for synthesis of silver NPs was demonstrated by physicochemical analyses such as UV-Vis Spectroscopy, whereas characterization of the Ag-NPs was carried out by X-Ray Diffraction (XRD), Transmission Electron Microscopy (TEM), Selected Area Electron Diffraction (SAED) and Energy Dispersive Spectroscopy (EDX) analyses. The biosynthesized Ag-NPs showed an antibacterial effect in planktonic cells of UPEC and also were effective to prevent the formation of UPEC biofilms. Thus, the contribution of this study was the establishment of the protocol for biogenic synthesis of AgNPs and characterization for its use as antibacterial agent to prevent biofilm formation of pathogenic bacteria.

Efficiency Comparison of Polyclonal Antibodies Against MDM2 Made in Rabbit and Chicken

Yolanda Rebolloso-Gómez, Victor H. Ojeda-Durán and Vanesa Olivares-Illana.
Laboratorio de Interacciones Biomoleculares y Cáncer. Instituto de Física, UASLP
Av. Manuel Nava No. 6 Zona Universitaria, San Luis Potosí. 78290, México.
Phone: 4448262362. yolandar@ifisica.uaslp.mx; victorojeda06@gmail.com

MDM2 is an oncoprotein of great biological relevance due to its role as the main regulator of the p53 tumour suppressor. p53 is activated in response to different cellular stresses that leads to several changes in the expression of a huge number of genes, related with the control of different cellular functions, such as, cell cycle arrest, DNA repair, senescence or even apoptosis. Commercial polyclonal antibodies are expensive and not very specific. Here we show the overexpression and purification of MDM2; from residues 1 to 301 and other from residues 322. After the His tag was removed, the protein was immunized in the chicken and rabbit, to produce polyclonal antibodies. Finally, will be tested different assays as Western Blot and inmunoprecipitation to compare their efficiency and specificity.

Heterologous expression of an antimicrobial peptide generated by bioinformatic analysis of anurans sequences

Diana Laura Resendiz Torres[1], Prisciluis Caheri Salas Navarrete[1], Lidia González Morales[1] y Luis Caspeta Guadarrama[2].
[1]Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos.
[2]Instituto de Biotecnología, Universidad Nacional Autónoma de México. Av. Universidad No. 1001, Col Chamilpa, Cuernavaca, Morelos, México. C.P. 62209. Tel. 777 3297057.
Email: lidia.gonzalez@uaem.mx

The inappropriate use of antibiotics in the treatment of infectious diseases has led the expansion of resistant bacteria. One alternative to face with this problem is to search for new therapeutic agents. A source for these compounds is the secretions of anurans skin, which are enriched with antimicrobial peptides; several of these have been isolated and characterized. Currently thousands of sequences of antimicrobial peptides from natural and synthetic origins can be found in databases like the Antimicrobial Peptide Database (APD). In this work, we collected information from anurans and analyzed with an algorithm written in Python, which uses ClustalW2 trough the matrix of substitution Blosum 62 and the structure analyzer in I-Tasser server. With this strategy, we generated a semisynthetic peptide of 25 amino acids with a potential antimicrobial activity. This was synthesized and cloned in the expression vector pQE-30, using the site directed-mutagenesis strategy. The antimicrobial peptide was expressed as a fusion protein with a histidine tail for subsequent purification in a nickel-agarose column. The antimicrobial activity of this peptide is further tested.
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In vitro probiotic assessment of *Pantoea dispersa* isolated from aguamiel and pulque

Allison Vianey Valle Bravo, José Luis Rivera Corona
Universidad Politécnica del Estado de Morelos
62550, (777) 2293533, jrcorona@upemor.edu.mx

The presence of beneficial microorganisms known as probiotics, provides beneficial effects to consumer health, improving the balance of intestinal host, and reducing the risk of gastrointestinal diseases. Probiotics indiuces a improvement of nutrient assimilation, systemic immune and / or anti-inflammatory activities. Some studies have shown that probiotics can play an important role in the formation,growth and suppression of tumors, having antitumor effects. Most probiotics belong to the genus *Lactobacillus*, but *Bifidobacterium*, *Bacillus*, and *yeast* are also found.

Aguamiel is a beberage extracted from the sap of Agave secies. Fermentation of aguamiel produces pulque, a Mexican traditional alcoholic, fermented beverage. A regular consimption of small quantities of pulque, It's associated with various health benefits. Pulque consumption for the treatment of gastrointestinal disorders and intestinal infections can also be explained by the possible probiotic activities associated with the presence of diverse lactic acid bacteria such as *Leuconostoc citreum, L. kimchi. L. Mesenteroides* and *Lactobacillus acidophilus* detected in fresh sap and during pulque fermentation. The aim of this work is to evaluate aguamiel and pulque from Morelos state, as a potential source of microorganisms with probiotic potential equivalent to that of strains that are already on the market.

Methodology. Samples of aguamiel and pulque were collected from the state of Morelos. The isolation of the strains was carried out in MRS medium. For the isolated strains, the coagulation and acidification capacity of the milk was determined, and the catalase activity tests were carried out. Viability under hostile conditions (acid pH and bile salts 2%), growth at different temperatures, production of exopolysaccharides and inhibition of enteropathogens (*E. coli* and *S. typhi*) were determined. The tests were also performed on strains of L. casei shirota, B. clausii that are shown as probiotics in the market.

Results. Three strains were isolated with the ability to inhibit the growth of enteropathogens, which also proved to be viable after being exposed to environments that simulate gastrointestinal conditions. The three strains showed production of exopolysaccharides, and were able to acidify and coagulate milk, in addition to growing at different temperatures and presenting positive catalase activity. The microorganism that presented the highest probiotic potential, even higher than that observed for *L. casei shirota* and *B. clausii*, was identified molecularly as *Pantoea dispersa*. The rest of the strains of interest have not yet been identified molecularly, but due to their morphology and the result of biochemical tests, it is presumed to be *Leuconostoc spp.*

Desing of a system of temporary immersion of *in vitro* cultures of plants by gravity at laboratory level.

[1]José Ramón Rodríguez Vázquez, [1]Juan Carlos Rodríguez Sierra, [1]Luis Enrique Murillo Yáñez, [1]Lisset Herrera Isidrón.
Unidad Profesional Interdisciplinaria de Ingeniería Campus Guanajuato, Instituto Politécnico Nacional, C.P. 36275. Silao de la Victoria, Gto, México.
Corresponding author. jrrv117@gmail.com, lherrerai@ipn.mx (4731620453)

There are currently several different temporary immersion bioreactors. In particular,the RITA® bioreactor has a pneumatic immersion mechanism that requires auxiliary equimment, this can be a great inconvenience due to the associated cost of operation; therefore, a new immersion system is proposed which requires potencial energy to transfer the culture medium from one container to another, that is to say, movement by gravity effect. The main idea of the design is to move the fluid from a mobile tank to a static container with plants to generate immersion by height change and vice versa. To study this dynamic phenomenon, it was proposed to vary the diameter of the connection hose and the properties of the fluid (density and viscosity) in order to characterize the immersion velocities and use the most suitable configuration. The system requires a very low investment cost, however, an operator is needed to perform the dives, this makes it a bit dependent, however, the proposal is a great alternative for this type of system. It is recommended to use materials that meet the necessary conditions for *in vitro* cultures to have a system that is versatile, functional, low cost and esay scalling up.

Coupling Planar Chromatography Directly to Ambient Ionization Mass Spectrometry (AIMS) for Phytochemical Profile Analysis

Nancy Shyrley Garcia-Rojas, Jorge Molina-Torres, Robert Winkler*
CINVESTAV Unidad Irapuato, Department of Biotechnology and Biochemistry, Km. 9.6 Libramiento Norte Carr. Irapuato-León 36824 Irapuato Gto., México Tel: +52 (462) 62396 00 Fax: +52 (462) 624 58 46
shyrley.garcia@cinvestav.mx

Phytochemical profiles of plants are useful to identify molecules which have resulted in great importance in different industries as food, medicine or agriculture1. Analytical techniques for this purpose have been greatly improved as Chromatography and Mass Spectrometry (MS). Thin Layer Chromatography (TLC) is one of the most convenient tools to study compounds because of it is an inexpensive and fast chromatographic method for separating complex mixtures. In order to identify the molecules MS is a very informative technique, but laborious sample preparation is required, which reflects on time and money. Some recent approaches to couple these two techniques have been achieved with different ionization techniques, e.g., Matrix Assisted Laser Desorption Ionization (MALDI) or Desorption Electrospray Ionization (DESI), but they are expensive[2].
In this Sense, Low Temperature Plasma (LTP) ionization is an inexpensive easy to build and use ambient ionization source. In this work we present the coupling of TLC- LTP for direct analysis of phytochemical profiles, the incorporation of a continuous laser helps improving compounds desorption (LD) from the TLC plate.
We have already achieved the coupling of TLC-LD-LTP system for the analysis of standards by MS without prior treatment. Now we are working on phytochemical profiles determination and validation parameters. The complete system provides a low-cost tool for chemical characterization of plants. With this system it will be possible other determinations like analysis of storage stability, quality control or beverage quality.

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"Cloning and expression of the *fur* GDI_1248 gene of *Gluconacetobacter diazotrophicus* Pal 5"

Roldan-León B. E[1]., Martínez-Morales L. J[1]., Eugenia-Baca B[2]., Soto-Urzúa L.[1*]
Laboratory of Microbial Physiology of the Interaction Microorganism-Host1. Laboratory Interaction Bacteria-Plant[2]. Center for Research in Microbiological Sciences CICM-ICUAP. Benemérita Universidad Autónoma de Puebla. Ed. 103C B, Ciudad Universitaria, Av. San Claudio, Col. San Manuel, Pue. Puebla, México CP: 72570. bren.roldan249@gmail.com, lucía.soto@correo.buap.mx

Introduction: *Gluconacetobacter diazotrophicus* Pal 5 is an endophytic bacterium that promotes plant growth (BPCV). Iron is an essential element for the development of most bacteria, it is involved in very important cellular processes, the paradox of this metal is that it presents strong toxicity in high intracellular concentration for bacteria, the uptake of iron sources requires tight regulation, this regulation process is performed by Fur and Fur homologues. Bertalan *et al*., 2009 completely sequenced the genome of *G. diazotrophicus* Pal5, *in silico* analysis revealed the presence of two possible fur genes $GDI_{\_1248}$ and $GDI_{\_1398}$.

Objectives: To clone and express the *fur* gene $GDI_{\_1248}$ in the pEXP5-CT vector and purify the recombinant $FurGDI_{\_1248}$ protein and subsequent oligomerization analysis against divalent metal ions, oxidizing agents and reducing agents.

Methodology and Results: Homologous oligonucleotides *fur*$GDI_{\_1248}$ gene were designed, amplified by PCR and the expected fragment size of 534 bp was cloned into pEXP5-CT that adds a fragment of 6His in the C-terminal, to generate pEXP5-CT:*fur1248* was transformed into *Escherichia coli* Top 10, the transformants were analyzed by PCR and digestions with *Ava*I enzyme (fragments of 2767 and 451 bp), BLAST and Clustal W programs confirmed 100% of identity of the sequence. For protein expression of pEXP5-CT:*fur*1248 was transformed into *E. coli* BL21(DE3)pLys, were induced for *fur* expression, by adding different concentrations of IPTG at different times and temperatures, analyzed by 15% SDS-PAGE, and Western blot with antibodies monoclonal antipolihistidine.

Conclusions: The *fur*$GDI_{\_1248}$ gene was cloned and expressed at a temperature of 37 ° C, at times of 120 minutes and concentration of 0.1mM IPTG, this allows to have the optimal conditions for the purification of the recombinant protein $FurGDI_{\_1248}$.

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Development of a new viral vector system for overexpression of recombinant proteins of pharmaceutical interest in *Chlamydomonas reinhardtii*

Alejandro Romo-Avalos[1], Karen Lizbeth Reyes-Barrera[1], Jesús Aarón Avalos-Calleros[1], Ángel Gabriel Alpuche-Solís[1*] and Gerardo Rafael Argüello-Astorga[1*].
[1]División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica, A.C., IPICYT. Camino a la Presa San José #2055, Lomas 4a. Sección 78216, San Luis Potosí, S.L.P. México. Tel: 8342000
*Corresponding authors: alpuche@ipicyt.edu.mx; grarguel@ipicyt.edu.mx

The use of viral vectors for the overexpression of recombinant proteins in different platforms (mammal cells, insects, plants) has become a field of opportunities with possibilities in the transformation process optimization, in order to render higher yields. The utilization of microalgae as a platform for the production of proteins of pharmacological interest is a promising possibility, not only because of the high yields achieved but also because green microalgae such as *Chlamydomonas reinhardtii* are recognized as safe for human consumption (GRAS), hence diminishing the costs associated with the purification of recombinant proteins for biomedical applications. The main objective of this work is to develop a vector system derived of geminiviruses which would be able to express the gene of interest (GOI) at very high levels by integrating it into the genome of *C. reinhardtii* (via *Agrobacterium tumefaciens*) and its subsequent excision and multiplication as an episome. The design of the vector includes the fusion of a promoter (containing the viral origin of replication, Ori) fused to the gene of interest, which is flanked at its 3´end by an additional copy of the Ori. To promote the excision and replication of the synthetic episomes containing the GOI, the vector also includes an expression module to provide the virus replication protein in *trans*. We are currently testing the Rep and CP promoters of three tomato-infecting geminiviruses, fused to the GUS and GFP reporter genes, optimized in codon usage for *Chlamydomonas.* The constructs were subcloned into pChlamy_1 and pCAMBIA 1300 vectors. We will present the best constructs based on the promoters efficiency assessed by histochemical, fluorometric and flow cytometry analyses. The presence of recombinant protein will be confirmed by ELISA and Western blot assays.

Identification of pigments in strains of Pycnoporus

[1] Leonardo Romo Patiño, [2]Ma. de Lourdes Acosta-Urdapilleta,[3]Elba Villegas.

[1]Facultad de Farmacia, [2]Centro de Investigacion en Biología, [3]Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos. lello.romo@gmail.com

Introduction. *Pycnoporus* belongs to an edible group of fungus called Basidiomycetes. This basidiomycete has a characteristic red-orange coloration due to the pigments it produces, Cinnabarine, tramesanguine and cinnabarinic acid. These pigments have diverse properties such as: antioxidant, anti-inflammatory, antibiotic, antiviral and insecticidal. Cinnabarine is considered a natural alkaloid and phenoxazine due to its three aromatic ring structure which provides cinnabarine with antimicrobial properties and Biotechnological applications such as colorant, antiviral, acaracid and nematicid. Cinnabarine extracts have greater effect on Gram + bacteria, this has been demonstrated trough bioassays that indicate activity against *Salmonella typhi* and *Staphylococcus aureus*. We have a collection of *Pycnoporus* of strains in the ceparium HEMIM of CIB-UAEM, which were collected from different parts of Morelos State. Our main objective is to determine and select the best *Pycnoporus* strain out of our collection, basing on the speed of growth and the production of pigments. Materials and Methods. Mineral and HIT agars were used to cultivate the strains at a pH of 5.5 in Petri dishes. Radial growth was measured for a period of seven days. Extracting the mycelium of each strain until 500 mg/strain were collected, then exhaustive extraction with acetone were carried out. Once extracted acetone was evaporated and the samples were suspended in HPLC-grade water. The absorbance of each extract was measured at 435 nm, then the concentration of Cinnabarinic acid, cinnabarine and tramesanguine were quantified using a standard curve and the molar extraction coefficient for 18,000Mcm^{-1}. Each extract was purified by HPLC. Results demonstrate that strains HEMIM- 52, 53, 61, 65, 69, 70, 72 and 78, produce higher concentracions of pigments among the other strains, also they possess the highest rate of growth. In the ther hand strains HEMIM-61 and HEMIM-65, have the highest yield of cinnabarine production. Conclusions. To growth these strains in Petri dishes with agar allow us to obtain enough mycelium, this method allowed us to growth the strains very fast in 7 days, extract the pigments in acetone, concentrate them to be quantified by HPLC.

Design of a Chromogenic Culture Medium based on Nejayote Water for the Identification of *Candida albicans* involved in Vaginal Candidiasis.

Tayde Sepori Salgado Hernández and Elba Cristina Villegas Villarreal Centro de Investigación en Biotecnología, UAEM. Av. Universidad No. 1001, Col. Chamilpa, 62210. Cuernavaca, Mor. Fax: 7773297030. Correo: elbav@uaem.mx.

Vaginal candidiasis is a health problem in Mexico, that is not of mandatory report, however it is of undoubted importance due to its frequency in women mainly in gynecology cases where it is present in 80-90% of all cases and because the difficult of the treatment, since doctors prescribe fluconazole without identifying the yeast responsible for the infection or its resistance to fungicides. Although other species are also detected but with less frequently for instance *C. tropic*alis and *C. grablata* the treatment is the same. Most women will have an infection of this type at least once throughout their lives. In addition, half of them will present at least two or three infectious episodes in a year, that is why vaginal candidiasis is considered a universal problem, affecting millions of women in tropical and subtropical zones. Candidiasis has also emerge as a hazard disease in immunosuppressed patients such as, in AIDS, HIV or those receiving anticancer agents or antibiotics. These types of infections are usually diagnosed by clinical signs and symptoms accompanied or not by a direct examination of the vaginal discharge but without the proper confirmation of mainly *Candida albicans*. In this work, a chromogenic selective and differential liquid and agar medium was designed to identify *Candida albicans*, based on Nejayote water. Nejayote water is produced as a water waste from traditional or industrial tortilla production (nixtamalization residue). It is based on a standarized nejayote water composition for glucose, total sugars, amino acids, protein content at a final pH value. The chromogenic substrate in this medium is directed to identiify the enzymatic activity of N-acetyl-D-glucosaminidase of *C. albicans*. The advantages that offer are: improved sensitivity and specificity, easy to use and to interpretate results, cost-effectiveness and the possibility to re-use Nejayote water aking protfits. The medium was validated with 50 clinical isolates samples from patients with vaginal Candidiasis, there were obtained from the Family Medicine Clinic of the ISSSTE of the Morelos State and using comercial CHROMagar candida to validate results. The nejayote water was also evaluated as a water waste following the Mexican norms “NOM-OO1-ECOL-1996“ and “NOM-CAT-031-ECOL/1993”, they establishes a maximum permissible limits for contaminants in waste water discharges in national waters and goods, determining total nitrogen, electrical conductivity and biochemical oxygen demand amng ther parameters. In the new Chromgenic Nejayote media *C. albicans* grew forming blue colonies, *C. glabrata* grew forming creamy pink colonies it was used as t negative control. the designed medium was 98% efficient in identifing *Candida albicans*.

Physiological and molecular characterization of *Saccharomyces cerevisiae* strains for industrial Wine production

Cindy Zuleyka Sánchez Arias, Naurú Idalia Vargas Maya, Gloria Angélica González Hernández, Adriana García Tapia, Israel Enrique Padilla Guerrero and Juan Carlos Torres Guzmán*.
Departamento de Biología. División de Ciencias Naturales y Exactas. Universidad de Guanajuato, Campus Guanajuato, Noria Alta s/n, Colonia Noria Alta. Guanajuato, Gto., México. C.P. 36050. Tel. (473) 7320006 ext. 8160. Email: torguz@ugto.mx

The wine industry has an essential influence in the development of the economy of several countries; this alcoholic beverage is produced by the fermentation and aging of different types of grapes. There are many varieties of wine, which depends on the type of the raw materials and the processes. The value of each wine type depends on the integrity of the flavors produced by the grape must, which are preserved and developed in each treatment of the process. The yeast *Saccharomyces cerevisiae* is the yeast most commonly employed in the wine fermentation process, for this reason, it represents a pivotal element to study.

Our goal is to obtain strains of *S. cerevisiae* with higher capacity and flexibility to adapt to adverse conditions. The development of these new strains represents an improvement in the process of winemaking, which implies a lower production cost and a better final product.

To make a successful fermentation is necessary to create an optimal environment for *S. cerevisiae*. The most important conditions are a percentage of grape must, percentage of ethanol of the end of the process, temperature, osmotic and oxidative stress. Each strain of *S. cerevisiae* has a different tolerance range. We studied the conditions mentioned in 3 different strains: the first strain used in the production of white wine, the second one is for red wine production, and the last one is a control strain. Our study starts with the molecular characterization of each strain. PCR amplification, cloning, and sequencing of the ITS (internal transcribed spacers) regions; amplification of microsatellites, inter delta regions and species-specific genes like beta-tubulin, SB, and SC. In the physiology characterization, we analyzed the range of living conditions that our yeast can endure. Posteriorly, we obtain our new strains by making an artificial selection: we grow the strains mentioned before in comfortable conditions; we then change the conditions to a hostile environment subtly. Once we obtain our new strains, we repeat the physiological and molecular characterization. We expect the new strains to develop new physiological characteristics with improved fermentative capacity.

Endophytes non- rhizobiales isolated from nodules of *Mimosa pudica* with biotechnological potential

Ricardo Sánchez Cruz, Irán Tapia Vazquez, Jorge Luis Folch Mallol, Arnoldo Wong Villarreal, Universidad Autónoma del Estado de Morelos, Centro de Investigación en Biotecnología, Cuernavaca, Morelos. C. P. 62209, rsan9207@gmail.com

Keywords: Endophyte, non-rhizobiales, PGPB, legumes.

The legumes have the ability to make symbiosis with different microorganisms, many nude bacteria are considered plant growth promoting bacteria (PGPB), due to their ability to stimulate the growth of plants, through direct and indirect mechanisms.
There are several reports of non-rhizobial endophytic bacteria with the ability to stimulate plant growth (Zaheer et al., 2016), so in this work we evaluated the capacity of 10 isolated Mimosa pudica nodules, with analysis of the 16s gene were identified belonging to the family Enterobacteriaceae and Results of the characterization of their ability to promote plant growth (production of indole acetic acid, phosphate solubilization, siderophore production, antagonism against phytopathogens) showed that they have several applicable characteristics to be BPCV, in addition 2 isolates identified as Enterobacter sp. have the presence of the *nif*H1 gene. The effect of the isolates on the growth of bean plants was evaluated, although all the isolates have characteristics of PGPB, only some had beneficial effects in the growth promotion of bean plants compared with the controls (nutrient solution and water), likewise the type of interaction that exists between one of the isolated NOD5 (Enterobacter sp.
with the presence of the *nif*H1 gene) and the roots of bean plants was studied, the ability to colonize nodules in the presence of a rhizobial bacterium was demonstrated (*Rhizobium etli*) but the ability to form nodules and fix nitrogen was not demonstrated.

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Molecular characterization of fructansucrase of *Clavibacter michiganensis* subsp *michiganensis*

[1]Azalia Sánchez Cruz, , [1]Luis Gerardo Quintanilla,[1]Jesús Hernández Romano, [2]Clarita Olvera Carraza y [1]Sandra Morales Arrieta
[1]Universidad Politécnica del Estado de Morelos, Boulevard Cuauhnáhuac No. 566 Col Lomas del Texcal Jiutepec Morelos.
CP 62550, 16120619@upemor.edu.mx 7775637742

INTRODUCTION Bacterial cancer is one of the main fitopathogenic problems that affect tomato culture, caused by the worldwide distributed bacteria *Clavibacter michiganensis* susbps *michiganensis.* It is one of the most devastating tomato culture diseases, causing losses up to 70% of tomato culture [1]. It has been reported that exopolysaccharides could cause water stress, therefore, be responsible for the development of wilting in tomato plants. However, it has been shown that exopolysaccharides are not a crucial pathogenicity factor. Exopolysaccharides produced by *Clavibacter michiganensis* susbps *michiganensis* are not directly involved in virulence, a strain with mutant phenotype that does not produce EPS, this does not present colonization or symptoms of plant tissue [2].
On the other hand, it has been reported that fructans (fructose polymers) generate plantmicroorganism
interactions [3]; where the fructansucrases are responsible for synthesizing these polysaccharides. In our work group a fructansucrase of *Clavibacter michiganensis* susbps *michiganensis* was bioinformatically identified, so our objective is to show that the hypothetical gene CMM_0614 codes for a fructansucrase.
METHODOLOGY. Primers were designed with NcoI and Apa I restriction sites. In order to isolate the fructansucrase gene, chromosomal DNA from *Clavibacter michiganensis subsp michiganensis* was purified and used as a template with Gotaq polymerase to amplify a PCR fragment, this product was cloned into the pBAD vector in *E. coli* Top10. The correct construction of the plasmid was confirmed by sequence analysis of single DNA strands from the insert. Positive transformants were selected and the optimal expression level was performed.
RESULTS AND DISCUSSION.
Blast analysis of fructansucrase of *Clavibacter michiganensis subsp michiganensis* showed that it has an 55 % identity with *Gluconacetobacter diazotrophicus* PA1, 38% with *Erwinia amylovora* CFBP1430 and 32 % with *Bacillus subtilis* subsp *subtilis* str 168. The amino acid sequence analysis revealed a signal peptide of 31 amino acids that was predicted with (Signal P 4.1). A model of the structure of the enzyme showed β-propel type, with five sheets that adopt a “W” topology of four β-antiparallel strands. The amplified gene codes for an enzyme of 52.2 kDa and its isoelectric point is 4.78.
The gene encoding for the levanase was amplified by PCR using specific primers, the product of 1566 bp amplified was cloned and sequenced.
CONCLUSIONS Fructansucrase sequence was identified from the genome of *Clavibacter michiganensis* subsp *michiganensis* (CMM_0614). Fructansucrase of 52.2 kDa and 547 amino acids has a high identity with the glycosil hydrolase 68 family en the active site. Conditions to express the protein had been established.

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Preparation of nanostructures with controlled architecture by the self-assembly of designed DNA and proteins.

Eddie Guillermo Sánchez-Rueda; Armando Hernández-García
Instituto de Química UNAM. C.P. 04510. Tel. (55) 56224548; eddie.sanchezr@gmail.com

In the recent years an increase in the research for the obtainment of nanostructures using biomolecules as construction blocks has taken place, allowing the development of a new area known as bionanotechnology. DNA is an excellent building block at the nanoscale, it is due to its specific three-dimensional organization, predictable base-pairing and self-assembly among several strands.

This project aims to obtain bi-dimensional (2D) and three-dimensional (3D) nanostructures using DNA and proteins programmed to interact in a precise manner. The 2D structures were designed in a way that short ssDNA molecules would assemble to form a small branched construction. To increase the size of the arms, long dsDNA (732 bp) was obtained via PCR, in which a triethylene glycol spacer molecule was incorporated to the primer to allow the formation of sticky ends; thus, dsDNA would be able to hybridize to the extremities of the small branched structure to form long arms. A second approach for the 2D structures took advantage of the high affinity between biotin and streptavidin, in such a way that, when using biotinylated dsDNA, constructions with three and four branches were produced. Both strategies generate products that can associate to the M13mp18 plasmid (ssDNA), generating 3D structures with a tetrahedral conformation.

It is well known that DNA nanostructures lack rigidity, which hinders the obtainment of defined architectures. To face this problem this project proposes the covering of the nucleic acids with three protein-based polymers, containing a block that binds to DNA through nonspecific electrostatic interactions. These ones have been previously reported by our research team and are called C4-B^{K12}, C_4S10-B^{K12} and C_8-B^{Sso7d}.

Production of the bi-dimensional constructions has been evaluated by a series of electrophoretic analysis. The nanostructures with three and four branches, obtained with both strategies, were successfully characterized by atomic force microscopy (AFM), verifying the expected organization of the molecules. However, the arms' length was notably irregular, as the DNA presented several flexions. The characterization of the constructions covered with the proteins is still pending, but it is expected to present a homogeneous distribution of the size and topology. Thus far, the interaction between the protein-based polymers and the DNA nanostructures has been demonstrated by electrophoretic mobility shift assays (EMSA).

Development of a nanocomposite with antibiofilm activity and permissive for delivery of Mesenchymal Stem Cells

Sánchez-Sánchez, Roberto[1]., Pérez-Díaz, Mario., Silva-Bermudez, Phaedra., Martínez-López, Valentín., Melgarejo-Ramírez, Yaaziel., Ibarra, Clemente., Martínez-Pardo, M. Esther (ININ)., Velasquillo, Cristina., Martínez-Castañon, Gabriel (UASLP) and Martinez- Gutierrez, Fidel (UASLP).*
[1]. Unidad de Ingeniería de Tejidos Terapia Celular y Medicina Regenerativa, INR LGII, PC 14400 Mexico City. 59991000 x 19604 sanchez2.roberto@gmail.com

Introduction. Wound healing is a process that is often affected by bacterial infection; the indiscriminate use of antibiotics has generated multi-drug resistant bacteria, complicating proper wound reparation. In this sense, silver nanoparticles (AgNPs) appear as an alternative to antibiotics. Cover the wounds from the environment is crucial to avoid infections and prevent loss of water; Radioesterilized Pig Skin (RPS) is a dressing that has been used for these purposes. Mesenchymal Stem Cells (MSC) have been proved to help wound healing by releasing growth factors, differentiate to fibroblast, promote vascular tissue formation and regulating inflammation. For these reasons, it was developed a RPS-AgNPs nanocomposite with antibiofilm activity and also permissive for growth of MSC.
Materials and methods. We performed the synthesis of AgNPs that were characterized by TEM and DLS. RPS was impregnated with different AgNPs concentrations and these nanocomposites (NC) were characterized by SEM and EDX. Antibiofilm effects of the NC were tested by colony biofilm model and Kirby-Bauer assays. Then MSC were characterized by flow cytometry and seeded on the NC. Cell viability was determinate by calcein assays and proliferation analyses were performed by cell proliferation curves.
Results and discussion. AgNPs showed spherical shape and presented a 10 nm size as measured from TEM and DLS; zeta potential values were -38±8 mV. These AgNPs showed better bactericide effect than antibiotics against Gram positive (21.3 folds) and Gram negative bacteria (16.2 folds) strains isolated from burned patients; bactericide effect was determined by minimum inhibitory concentrations. As it was expected, EDX and SEM assays showed higher Ag concentration in NC impregnated with more concentrated AgNPs solutions. NC showed inhibition zones for gram + (G+) and – (G-) bacteria compared with RPS without AgNPs which did not show inhibition zones.
NC displayed antibiofilm properties at 250 ppm AgNPs solutions for G+ and 1000 ppm for G- bacteria. The MSC were positive for characteristic MSC surface markers measured by flow cytometry and were capable to survive on NC impregnated with 250 ppm AgNPs solutions, with a reduction of 35% of viability in comparison with the control. MSC decreased in number when were seeded on NC but on NC impregnated with 125 and 250 ppm AgNPs solutions, MSC proliferate.
Conclusions. It was developed a construct with the potential to promote wound healing and the capability to prevent bacterial growth, and consequently that could contribute to restore damaged skin tissue.

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Generating a diagnostic method for differentiation between Zika and Chikungunya virus by PCR, using samples Tabasco State

Santos Fernández Ingrid Araceli[1], López Mondragón Daniela Alejandra[1], Landa Pineda César Manuel[1,2], e-mail: ingriidsfd@gmail.com,
[1]Lic. Químico Farmacéutico Biólogo. [2]Maestría y Doctorado en Química Aplicada. [3]Laboratorio de Biología Molecular y Farmacogenómica. Universidad Juárez Autónoma de Tabasco. División Académica de Ciencias Básicas. Carretera Cunduacán-Jalpa KM. 1 Col. La Esmeralda CP. 86690 Cunduacán, Tabasco, México.

The Zika virus is an arbovirus to the genus Flavivirus, it is transmitted into human by the sting of the *Aedes aegypti* mosquitoes and causes the disease that bears the same name; patients with this disease present a clinical picture that includes, fever, conjunctivitis, muscular and joint pain, headache, which lasts approximately 2 to 7 days.

Another disease that presents a similar clinical picture is Chikungunya fever, this is a viral disease transmitted to humans by the mosquitoes *Aedes aegypti* and *Aedes albopictus*, which are carriers of the virus, its genome is RNA and belonging the genus Alpha virus, Family Togaviridae. The word "Chikungunya" comes from the Kimakonde language that means "bending", which refers to one of the main symptoms in patients, such as muscular and articular pain. Both pathologies have similar clinical pictures, so their diagnosis is often confused, so it is necessary to perform a differential study to detect the presence of one of the viruses through antibodies, since diagnosing based on the clinical picture is unreliable

There is not specific treatment for these illnesses rather it is to alleviate the joint and muscular symptoms using optimal or liquid analgesics, there is currently no vaccine that can remove any of these viruses. Drugs that should be avoided are acetylsalicylic acid and anti-inflammatory because they can alter blood clotting.

The contagion of these two viruses mainly affecting the tropical and subtropical regions, because the environmental conditions are optimal for the propagation of mosquitoes transmitting these viruses. These conditions include high level temperature and precipitation. Before 2013, the virus was found in Africa, Asia, Europe, the Indian Ocean and the Pacific Ocean. At the end of 2013, shoots were presented for the first time in Latin America and the Caribbean. In Latin America local transmission of the disease has been found in 44 countries and territories.

This work is intended to analyze the complete genomic sequences through multiple alignments of both species to design first to identify and differentiate, by PCR, using samples of patients with symptomatology Concerning an infection by either Zika or/and Chikungunya.

Evaluation and microbiological Determination, organoleptic and physicochemical of beer production with wild yeast.

Álvarez Quiroz Ernesto, l Paz García Román Bruno, *Fuentes Ramírez Luis Ernesto, * Antonio Castañeda Dolores,* Avelino Flores Fabiola y *Santoyo Páez Yuriria.
Facultad de Ciencias Biológicas, Benemérita Universidad Autónoma de Puebla.
lLicenciatura de Biotecnología, Benemérita Universidad Autónoma de Puebla.
*Centro de Investigación en Ciencias Microbiológicas-ICUAP, BUAP, Puebla,
yuriria.santoyo@correo.buap.mx

Introduction: The Reinheitsgebot law (the law of purity) in Germany, which stated that the production of beer was only with three ingredients (barley, hops and water), nowadays a wide variety of ingredients are used that provide unique flavors to the wort, for example a diversity of wild or commercial yeasts, which dominate the production of alcoholic beverages worldwide (Walker et al 2016); particularly in the brewing industry. Mexico ranked fourth in beer production in 2017 with 110 million hectoliters and the first in exporting, with 33 million hectoliters worldwide, obtaining a profit of 3,768 million dollars. In recent years there has been a trend in the production of craft beer in Mexico, as 40 new breweries open each month in search of new styles, especially with the use of Mexican supplies (Industria alimenticia, 2016), and this is why it is very important the search for wild Mexican yeasts, which may allow brewers to innovate in this industry.

Objectives: Evaluation and microbiological, organoleptic and physicochemical determination of beer production with wild yeast.

Methodology: Twenty-one wild yeasts previously characterized by their colonial morphology, microscopic and biochemical tests were tested. A brewing recipe was designed that was used for the types of fermentation ale and lager, in order to identify the aromas and flavors that are released during the process with the wild yeasts and type strains. The beer was evaluated with physicochemical tests: esters and aldehydes identification, fixed acidity and determination of alcohol content. Organoleptic tests were carried out by a group of tasters and to evaluate the attenuation capacity of wild yeasts these tests were carried out: measurement of brix degrees and conversion of alcohol content. Finally, microbiological tests such as the detection of total coliforms based on the Mexican Official Norm (NOM) applicable to fermented alcoholic beverages were run.

Results: The fermentation time varied in comparison to the type strains, as they fermented in a week while the wild yeasts in 3 weeks as the minimum, behaving like a lager type yeast that may take from 1 to 3 weeks in its fermentation. Some of these yeasts obtained results close to the type, such is the case of yeasts 15, 37, 98 and 102, all in ale fermentation. In general, the yeasts tested for lager fermentation had a higher percentage of brix degrees, 6.86%, compared to the ale fermentation of 5.89%. In the organoleptic evaluation, strains 1 and 82 in the Ale style and for the lager style strains 37 and 15 obtained better results as they released better flavors and had a better acceptance among the tasting panel. Regarding to the physicochemical tests, most results have esters above the levels established by the Mexican Official Norm (NOM), however we, found that commercial beers similar to the style with high levels of esters; these measures depend mainly on the yeast used and the style. Finally, the microbiological tests allowed us to detect failure in the elaboration process since a considerable part of the samples were contaminated.

Conclusions: By testing and brewing wild yeast, we may say there is a collection of yeasts with potential for their use in the craft beer industry since the diversity in metabolites released during fermentation process opens a new panorama for beer in Mexico.

Topic area: Biotechnology

Arbuscular mycorrhizal symbiosis, growth and photochemical activity are affected by the phosphate concentration in *Stevia rebaudiana*.

Luis Gerardo Sarmiento López[1], Melina López Meyer[2], Gabriela Sepúlveda Jiménez[1], Luis Cárdenas Torres[3] and Mario Rodríguez Monroy[1]. [1]CeProBi, Instituto Politécnico Nacional, Carretera Yautepec-Jojutla, Km. 6, calle CEPROBI No. 8, Col. San Isidro, Yautepec, Morelos, México. CP 62731. 2CIIDIR-SINALOA, [2]Instituto Politécnico Nacional, Blvd. Juan de Dios Bátiz 250, Guasave, Sinaloa, México CP 81101. [3]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, México CP 62271. lsarmientol1100@alumno.ipn.mx

Key words: *Mycorrhizal symbiosis, Phosphate, Stevia rebaudiana*

Stevia rebaudiana is a perennial herb of significant economic value due its the high content of steviol glycosides (SG´s), natural sweeteners found in leaves. Arbuscular mycorrhizal (AM) symbiosis is a biotechnological strategy used in agriculture to reduce the use of agrochemical and may increase mineral nutrients, uptaker water, plant growth, biosynthesis of secondary metabolites and induce changes at the photochemical activity. It is well known that phosphate concentration plays an important role in symbiotic and nutritional development. Additionally, AM symbiosis can generate biotic stress, by modifying its photochemical activity. However, studies on these characteristics in *S. rebaudiana* under AM interaction are limited. The objective of this study was to evaluate the effect of phosphate on the AM symbiosis establishment, growth and photochemical activity in *S. rebaudiana*. Cuttings of *S. rebaudiana*, were procured from CeProBi-IPN, Yautepec, Mexico. The substrate used was a mixture of 1/1 sand-vermiculite, plants were inoculated with *R. irregularis* (250 spores per plant) (kindly provided by Dra. Melina López Meyer, CIIDIR-Sinaloa, Instituto Politécnico Nacional, Mexico) and fertilized twice weekly with half-strength Hoagland's solution containing 20, 200, 500 and 1000 µM PO_4^{3-}. Roots of *S. rebaudiana* were cleared and staining to determine mycorrhiza colonization percentage. The plant growth was measured in terms of its fresh weight of leaves, root, root length and photochemical parameters were measured using a portable fluorometer OS-30P. *R. irregularis* successfully established mutualistic symbiosis in *S. rebaudiana* roots, and high percentage (73.26 ± 17.90 and 67.01 ± 19.34) of AM was observed in phosphate concentration of 20 and 200 µM, respectively. However, it was found a significant decrease colonization (43.33 ± 4 and 20.37 ± 17.01) at phosphate concentration of 500 and 1000 µM, respectively. Structures such as vesicles, extra e intraradiculares hyphae and arbuscules was visualized by confocal microscopy. Fresh weight of leaves and roots was significantly different with respect to the phosphate concentration and AM colonization. The photochemical parameters of *S. rebaudiana* plants showed differences only in the treatment with 20 µM PO_4^{3-}, phosphate concentrations (200, 500 and 1000 µM) did not show differences between mycorrhizal and non-mycorrhizal treatment.

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Sub-cloning and heterologous expression of Mnn1 enzyme from *S. cerevisiae* in *E. coli*

Esteban Francisco Serrano, Arianna Núñez Beltran, Héctor Manuel Mora Montes, Juana Elizabeth Reyes Martínez*
Departamento de Biología, División de Ciencias Naturales y Exactas, Campus Guanajuato, Universidad de Guanajuato, Noria Alta S/N, Guanajuato, Gto. C.P. 36050. Tel: (+52) 473-7320006 Ext: 8158, e-mail: juana.reyes@ugto.mx

Abstract

Diarrhea remains as one of the leading causes of childhood death in low and middle income countries, in many cases ended in severe diarrhea and death in children under age 5, carrying consequences as high susceptibility to infections, low weight and malnutrition. Enterotoxigenic (ETEC) and enterophatogenic (EPEC) *Escherichia coli* strains are one of the main causal agents of diarrhea. Up to date, there is not an appropriate diarrhea vaccine to easily administrate against ETEC and EPEC strains. *O*-antigenic polysaccharides are part of the bacterial lipopolisaccharide and are frequently used for serological typing; those are located in the outer membrane of Gram-negative bacteria. EPEC serogrups O8, O9 and O9a have been described as linear mannose homopolymers.

The synthesis of *O*-antigens is important to investigate structure-bioactivity relationship among the oligosaccharides and its recognition proteins. Chemical synthesis of oligosaccharides carries several difficulties; uses hazardous and harmful chemicals and solvents, is time-consuming to structurally assemble complex carbohydrates, produces large amounts of toxic waste with low yields.

Enzymatic strategies using glycosyltransferases is a very attractive and greener strategy for glycan synthesis.

In order to create biocatalytic routes for the synthesis of *O*-mannosyl polysaccharides presents in ETEC and EPEC strains, cloning and producing enzymes with mannosyltransferase activity has been the main goal in this project.

The open reading frame of *Saccharomyces cerevisiae* α-(1→3)-mannosyltransferase was successfully sub-cloned in pJET1.2 vector to subsequently insert it into pET32a expression vector.

E. coli BL21 competent cells were then chemically transformed with the construct pET32a-MNN1. Optimum expression conditions were determined by SDS-PAGE at 8 hours after induction with IPTG [0.5 mM]. The protein present a 63 kDa size which is also correlating with His-tag detection, but the enzyme is remaining in the insoluble fraction. Purification was performed by Ni-NTA method and activity will be determined in the next weeks.

S. cerevisiae Mnn1 enzyme was successfully expressed in *E. coli* system, the enzyme will be integrated for ETEC and EPEC polysaccharides synthesis, this will allow to develop biotechnological tools to prevent *E. coli* related diarrheal episodes.

Deciphering the role of bean *RALF*, *FER* and *RIPK* genes in symbiosis with rhizobia

Jorge Solís-Miranda, Marco Juárez-Verdayes, Noreide Nava and Carmen Quinto.

Department of Plant Molecular Biology, Institute of Biotechnology, UNAM. quinto@ibt.unam.mx.

The Rapid Alkalinization Factors (RALFs) are a family of cysteine rich peptides that are well conserved in all land plants. These peptides have a role in regulating plant cell elongation (1). In *Arabidopsis thaliana* roots, RALF peptides interact with a malectine like receptor kinase FERONIA (FER), leading to the inhibition of a plasma membrane H+ ATPase 1 (AHA1), that results in the inhibition of the primary root growth (2). After RALF treatment, FER interacts with a cytoplasmic kinase RIPK1, and both kinases are then activated (3).

It is well accepted that some signaling pathways involved in response to pathogens, are shared with those in symbiosis (Zipfel y Oldroy). RIPK1 has been related to pathogen responses, therefore this urged us to analyze the role of this kinase as well of its interactor FER and also RALF, in the mutualistic association between legume roots and rhizobia. Earlier, it has been reported that the complex RALF-FER-RIPK, regulate polar growth in root hairs (Du et al). Combier et al., (2008) described that RALF is required for the establishment of an effective symbiosis between *Medicago truncatula* roots and *Sinorhizobium meliloti,* nevertheless the functional role of *FER* and *RIPK* has not been examined yet in symbiosis.

We evaluated the promoter activity of *RALF, FER* and *RIPK* genes in *Phaseolus vulgaris* roots in symbiosis with rhizobia, as well as their functional role during nodule organogenesis, by reverse genetics. Herein, the spatio-temporal activities of two *FER* (*PvFER1* and *PvFER2*), two *RALF* (*PvRALF1 and PvRALF6*) and two *RIPK* (*PvRIPK2 and PvRIPK3)* gene promoters from *P. vulgaris* were determined in hairy roots during nodulation. A clear promoter activity for each of the six genes studied was observed during the development of nodule primordia and in the nodule vascular tissue, suggesting the participation of these genes in nodule organogenesis. Over-expression and down-regulation of these individual genes is in progress to define their role during nodulation.

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Discovery of two bacterial type III polyketides with potential bioactive capacity from marine ecological niches

Arianna Soto-Hernández1, Omar Jiménez-Rodríguez[2], Holber Zuleta-Prada[3], Sergio Sánchez[2], Hugo Serrano-Posada[1,4] and Sara Centeno-Leija[1,4]‡
[1]Laboratorio de Agrobiotecnología, Tecnoparque CLQ, Universidad de Colima; [2]Instituto de Investigaciones Biomédicas, UNAM; [3]División de Ciencias Forestales, Universidad Autónoma de Chapingo; 4Cátedras CONACyT, Dirección Adjunta de Desarrollo Científico. ‡Corresponding author: S. Centeno-Leija, Consejo Nacional de Ciencia y Tecnología, Laboratorio de Agrobiotecnología, Tecnoparque CLQ, Universidad de Colima, Carretera Los Limones-Loma de Juárez, 28629 Colima, Colima, Mexico; Tel. +52 (312) 3161000; e-mail: scenteno0@ucol.mx

Keywords: bioactive compounds; bioinformatics tools; polyketide synthase; *Streptomyces*

Polyketides are secondary metabolites produced by eukaryotic and prokaryotic organisms, which commonly exhibit bioactive capacity. In bacteria, type III polyketides are synthesized by type III polyketide synthases (PKSs III), which catalyze the decarboxylative condensation of acyl-CoA monomers to generate β-polyketones such as stilbenes, chalcones and pyrones. Recently, the discovery of a huge library containing several putative PKS-III encoding genes in the genomes of microorganisms from “exotics” ecological niches, revealed the enormous potential to find novel bacterial bioactive type III polyketides. In this work, two putative bacterial PKSs III enzymes (PKS III-A and PKS-III-B) were detected by metagenomic analysis of a bacterial consortium from marine ecological niches. The bioinformatic analysis showed that PKS III-A and PKS-III-B enzymes could be involved in the production of alkylresorcinol- and aryl polyene-type polyketides, respectively. Using an integrative system ΦC31, we programed two wild-type *Streptomyces* strains (*S. coelicolor* and *S. venezuelae*), as well as an engineered *Streptomyces* strain (M1152) for the heterologous production of both hypothetical type III polyketides. Together, product characterization by NMR and bioactive capacity assays of both type III polyketides, as well as structural and biochemical studies of PKS III-A and PKS-III-B enzymes will offer an opportunity to gain insights into the biosynthesis of novel bioactive marine natural products.

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Partial characterization of *Scolopendra viridis* Say and *S. polymorpha* venom proteases.

Erika Duarte-Elguea, Lucero Valladares-Cisneros, Ma. del Carmen Gutiérrez-Villafuerte
Centro de Investigación en Biotecnología (CEIB), Universidad Autónoma del Estado de Morelos. Av. Universidad 1001 Col. Chamilpa C.P. 62209. Cuernavaca, Morelos. Tel: 01 (777) 3297057 Ext. 4556 y Fax: (777) 3297030 E-mail: carmengu@uaem.mx

SUMMARY

Increased interest in invertebrate venoms had resulted in the identification of a large number of enzymes with proteolytic activity (e.g. hyaluronidase, phospholipase A2, protease) (Malta *et al.*, 2008). Some of these enzymes possess properties which render them of medical and pharmaceutical interest. Interestingly, the study of these enzymes has led to the development of anti-inflammatory, clot dissolver and anti-retroviral drugs (Heinz, 2004; Müller-Esterl *et al.*, 2008). Despite the intensive study of scorpion, bee, wasp and spider venoms, there are some groups where little attention has been focused. Centipedes are one of the oldest arthropod groups that use venom as weapon to capture and digest their preys. However, the current knowledge of centipede venom composition is reduced. Therefore, the objective of this study is to partially characterize the proteases present in the *S. viridis* Say and *S. polymorpha* venoms. The proteolytic activity of both centipede species venom was evaluated by the turbidimetric assay. Zymograms were performed, by adding gelatin to the acrilamide gel, to locate the molecular weights of proteins with proteolytic activity. Proteins with enzymatic activity were recovered by the electroelution method. To estimate the optimal conditions of protease activity the turbidimetric assay will be carried out at different pH and temperature conditions. The results showed the presence of several low molecular weight (<30 kDa) proteins with proteolytic activity in the venom of both species.

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Activity of extracellular laccases of *Humpherella coffeatum* grown on different substrates

Benjamín Amaro Guadarrama[a], Alma Rosa Agapito-Ocampo[a], Gerardo Díaz-Godínez[b], Ma de Lourdes Acosta-Urdapilleta[a], Elba C, Villegas[c], Alexis Rodríguez[c], Maura Téllez-Télleza*.

[a]Centro de Investigaciones Biológicas. Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, México. *maura.tellez@uaem.mx.
[b]Centro de Investigación en Ciencias Biológicas, Universidad Autónoma de Tlaxcala, Tlaxcala. México.
[c]Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos.

Introduction: Laccases are blue-copper oxidases enzymes, have great industrial importance, in foods can be applied to certain processes such as to improve or modify the appearance and color of fruit juices, beers and wines by eliminating undesirable phenols. White-rot fungi are important producers of laccases, since they participate in certain biological processes, such as in the pigmentation of spores (Nigam, 2013), during the process of pathogenesis and during the formation of the fruiting bodies (Claus, 2004), for the internal detoxification of toxic components for the organism that can be formed during the degradation of lignin. In this work the laccase activity of *Humpherella coffeatum* grown on different substrates was determined.

Materials and methods: *H. coffeatum* (HEMIM-144) was grown in Petri dishes on 12 combinations of substrates based on pine, cedar, oak and bran. To obtain the enzymatic extract, 25 mL of water was added for each g of dry substrate invaded with mycelium, it was stirred in refrigeration for 18 h, after that the supernatant was recovered by filtration. The radial growth velocity (Vr, mm h^{-1}) and laccase activity using 2,6-dimethoxyphenol as a substrate at different pH's (3.5-6.5) were determined. One unit of laccase activity was considered as the amount of enzyme that increases one absorbance unit per minute and reported per g of dry substrate (U/g).

Results: On cedar and pine both 100% were observed the lowest Vr (0.013 and 0.014 mmh-1, respectively), the largest values of Vr were in combination of substrates, in pine and bran (90:10) the Vr was of 0.021 mmh^{-1}, with pine, cedar, bran (60:20:20 and 45:45:10) was 0.020 and 0.019 mmh-1, respectively. In general, the laccase activity presented values in descending order at pH 4.5, 5.5, 3.5 and 6.5. The highest laccase activity was observed in the following treatments: in the mixture of pine, cedar and bran (60:20:20) with 41.9, 44.9, 38.5, 4.8 U/g at pH 3.5, 4.5, 5.5 and 6.5, respectively. In the combination of cedar and bran (90:10) the activity was 25.2, 40.6 and 22.3 U/g at pH 3.5, 4.5 and pH 5.5, respectively; and in 100% cedar was 30.3, 38.4 and 43.2 U/g at pH 3.5, 4.5 and 5.5, respectively. The mixtures of pine, cedar, bran (45:45:10) and pine, oak, bran (45:45:10) showed lower activity at pH 6.5 than in the other pH, however, the other mixtures showed even less activity.

Conclusion: In general there was more activity in the presence of cedar, however there were differences depending on the pH, which could be due to the presence of isoforms, which can be corroborated by zymography.

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Growth of *Pleurotus ostreatus* in airlift reactor and stirred tank for laccase production

Luis Felipe Candia-Sánchez [1,2], Rubén Díaz[2], Maura Téllez-Téllez[3], Gerardo Díaz-Godínez[2*].
[1]Maestria en Biotecnología y Manejo de Recursos Naturales, Universidad Autónoma de Tlaxcala, Tlaxcala México.
[2]Centro de Investigación en Ciencias Biológicas, Universidad Autónoma de Tlaxcala.
[3]Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Morelos.
diazgdo@hotmail.com.

Introduction: The development at large scale of cultures has been based on the implementation of bioreactors such as airlift and stirred tank for the production of metabolites of interest. These techniques have allowed the establishment of the conditions for the cultivation of fungi. Pleurotus ostreatus is a fungus of great importance in the world, one of the most important aspects is its production of ligninolitic enzymes as laccases (1). These enzymes show a high catalytic capacity with potential biotechnological uses (2).
Methodology: *Pleurotus ostreatus* culture was performed in liquid fermentation in airlift bioreactor and stirred tank, both operated at 75% capacity. Each bioreactor with the culture medium was sterilized and inoculated with mycelium developed in PDA for 7 days, the initial pH of the culture medium was 6.5; the stirred tank reactor was operated at 120 rpm and in the airlift, the air flow was 1 vvm, both incubated at room temperature. The culture medium contained glucose, yeast extract and mineral salts (3). The sample taken from the airlift reactor was every 48 h and in the stirred tank every 24 h. Enzyme activity of laccase was determined in each sample using 2 mM 2,6-dimethoxyphenol (DMP) at pH 4.5 and 6.5. The absorbance was read in a spectrophotometer at 468 nm at one minute of reaction.
Results: Laccase activity in the airlift reactor was increased over time (obtained at 528 h, 3636 and 2972 U/L at pH 6.5 and 4.5, respectively). In the case of the stirred tank reactor, it was observed at 408 h, 4557 and 2956 U/L at pH 4.5 and 6.5, respectively.
Conclusions: *Pleurotus ostreatus* could be grown in airlift reactor and stirred tank obtaining different laccase activity values in each of the fermentations. The highest activity was obtained in the stirred tank reactor, possibly because in this cultivation system there is a greater oxygen solubility.

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ENTOMOCHEMICALS FROM *Pterophylla beltrani* AS BIOACTIVE COMPOUNDS FOR FOOD INDUSTRY

Jorge Ariel Torres-Castillo[1], Fabián Eliseo Olazarán-Santibáñez[1], Yolanda del Rocio Moreno-Ramírez[1], Reyna Ivonne Torres-Acosta[2], Arturo Mora-Olivo[1]
[1]Universidad Autónoma Tamaulipas-Instituto de Ecología Aplicada. División del Golfo No. 356. C.P. 87019. Ciudad Victoria, Tamaulipas. [2]Universidad Autónoma Tamaulipas- Unidad Académica Multidisciplinaria Mante, C. P. 89840,
Ciudad Mante, Tamaulipas, México.
jorgearieltorres@hotmail.com

Edible insects are consumed around the world in many cultures as complements for diets, they present high contents of proteins, fats and fibers. Currently, there are industries that generates food products based on some insect species, in fact, the consumption of insects has been promoted in last decades, considering them as part of balanced diet. However, several aspects (species, phenological stage, diet and others) must be considered before selecting the insect species, in order to ensure the nutritional quality of food products. Nutrimental contents are considered in food industry, but also the presence and activities of nutraceuticals has been considered as relevant in the last years, and with exploration of new food materials, the presence of nutraceuticals also must be studied. Here, the exploration of entomochemicals with nutraceutical potential from *Pterophylla beltrani* are presented. *P. beltrani* is an orthopteran that grows on foothills at the Sierra Madre Oriental in northeast Mexico, and during years it has been considered as potential candidate to be used as food for aquaculture and aviculture, but recently its relevance to be used a food for humans has been highlighted. Polyphenols and antioxidants have been studied using different solvents for extraction. It was observed that polar phenolics and antioxidants are the most abundant, because water, methanol and ethanol are the solvents with higher contents of such compounds in comparison with acetone and isopropanol. Statistical analysis indicated that water and methanol were the best solvents to extract antioxidant activities. Antioxidant activities from this insect were similar to antioxidant levels recorded from plants. An ethanolic fraction was analyzed for compound identification by mass spectrometry and results indicated the presence of alkaloids, phenolics, amino acid derivatives, organic acids and other; which, highlighted that antioxidant properties could be related no only to phenolics and that this could be produced by synergistic interaction. This insect showed potential to be consumed as food or to be used as material for food industry developments.

Characterization of *cadB1* a new gene involved in the degradation pathway of chloranilic acid in *Herbaspirillum* sp. strain TQ07

Luis Gerardo Treviño Quintanilla, Rosa Angélica Guillén Garcés, Thalía Oseguera Manzanilla, Itzel López Mendoza, Juan José Colín Salinas. Universidad Politécnica del Estado de Morelos, Jiutepec, Morelos, México, C.P. 62550, Tel. 7772293533. ltrevino@upemor.edu.mx.

The chlorinated aromatic compounds and their derivatives are persistent environmental pollutants used in the production of pesticides, pharmaceutical precursors, dyes and many other industrial products; however carcinogenic and chemical cytotoxic effects have been associated with many chlorinated compounds. Biodegradation is an important process that benefits the environment and helps reverse the pollution generated by human activities. The use of molecular techniques, proteomics and chemical analysis of metabolites have permitted to elucidate in detail the metabolic capacity that certain microorganisms have to remove toxic compounds by biodegradation. In this work, the genome of *Herbaspirillum sp.* strain TQ07 was completely sequenced and genes involved in the degradation were partially characterized by directed deletion. This strain use 2,5-dichloro-3,6-dihydroxybenzo-1,4-quinone (chloranilic acid, CA) as its only source of carbon and energy, this compound is a model molecule to study the degradation of aromatic compounds at molecular level. The analysis of the genomic sequence will allow us to characterize the region containing the genes involved in the degradation, if they show differences with microbial species of the same genus and the characterization of additional genes involved in CA mineralization. Starting from a set of Illumina™ sequencing reads, we assembled a 5,058,191 bp genome in 46 contigs, with 60.31 GC%. Genomic annotation was performed via RAST using the genus *Herbaspirillum* as reference, resulting in 4571 annotated features. The strain TQ07 spontaneously loses the ability to degrade CA when grown in a rich media; the mechanism of this phenotype was characterized as a recombination phenomenon that causes the loss of a genomic island of 120 kb, flanked by a repeated direct sequence of 51 bp and a homologous gene of the *xerCD* system at one of its ends. Also we found, that only *Burkholderia cenocepacia* strain DDS 22E -1 has a set of homologous genes similar in number, orientation and genomic distribution to TQ07 strain CA genes, differing only in that TQ07 strain has two additional genes, encoding a porin and a chemoreceptor, maybe involved in CA degradation. The construction of mutants by directed deletion of several genes presumably involved in the degradation of CA (*cadA, cadB1 and cadP*) allowed us to identify the gene *cadB1* whose homologs have not been previously involved in the degradation of chloroaromatic compounds, since *cadB1* mutant is unable to use CA as its sole source of carbon and energy. In addition there is a partial change of coloration in a solid culture medium supplemented with AC, which may indicate the accumulation of a metabolite

Expression of *Loos*1 from fungus *Bjerkandera adusta* in plants of *Arabidopsis thaliana* regulated by an inducible promoter to develop an auto-pretreatment protocol

Maria Magdalena Urzua-Abad1, Jorge Luis Folch-Mallol1, María del Rayo Sánchez-Carbente1
1Centro de Investigación en Biotecnología. Laboratorio de Biología Molecular de Hongos. Universidad Autónoma del Estado de Morelos.
Av. Universidad 1001. Colonia Chamilpa, C.P. 62209, Cuernavaca, Morelos. email: maria.sanchez@uaem.mx

The increase in the use of fossil fuels has generated global situations such as depletion in the reserves, an increase in greenhouse gas emissions, causing an impact on climate change which is a worldwide concern. In the last decades there is a search for alternatives to replace them, such as the production of biofuels like the bioethanol. A very promising source for bioethanol production is the use of raw lignocellulosic biomass, but due to its recalcitrance, it is necessary to perform pretreatments to deconstruct it in order to have access to the fermentable sugars. There are different pretreatment methods including the biological ones which are friendly to the environment. In the present work we are exploiring the use of amorphogenic proteins, as an alternative of biomass pretreatment. Amorphogenic proteins, such as expansins, swollenins and loosenin, can disorganize crystalline cellulose. Previously, our group have reported that loosenin isolated from *Bjerkandera adusta* has been shown to increase the release of sugars reducers in synergy with endoglucanases without the presence of hydrolytic activity using crytalline cellulose[1]. The aim of the present work is to express the loosenin heterologously in *Arabidopsis thaliana* plants to induce their loosening of the cell wall as a pretreatment previous to sacharification. To this work it is intended therefore to investigate the pretreatment in plant under controlled conditions and using an inducible system to carry out the saccharification. To achieve this, the expression of the loosenin will be controlled by an estrogen inducible promotor. We have cloned the loosenin and the GFP (as a control of the promotor activity) in the vector pMDC7 which presents a promoter inducible by estrogens2 using the Gateway technology , resulting in two constructions named pMDC7::GFP and pMDC7::PsCr-*Loos*1. We have carried out the transformation of *Arabidopsis thaliana* Col-0 with the method of floral dipping through *Agrobacterium tumefaciens*. The genotipification and experiments of inducible expression and sacharification are currently in process

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Efficient multiplication of *Vaccinium corymbosum* in temporary immersion systems (SIT)

[1] Miriam Isabel Vargas Avila, [2]Adanely de la Cruz Cruz, [1]Marina Vanessa Rougon Flores, [1]Juan Carlos Rodríguez Sierra, [1]Lisset Herrera Isidrón
[1] Unidad Profesional Interdisciplinaria de Ingeniería Campus Guanajuato. Instituto Politécnico Nacional. C.P. 36275. Silao de la Victoria, Gto. México. [2]TREO BERRY FARM. Tepatitlán, Jal. México.

Corresponding author: mairim2419@gmail.com, lissetupiig@gmail.com; 4771101045

Blueberry (*Vaccinium corymbosum*) is a species belonging to the genus *Vaccinium*, of the family *Ericaceae*. The main interest of it is exploitation is due to the growing commercial demand for it is fruits, used for fresh consumption, in juices, jams, etc., as well as in the pharmaceutical industry, due to it is high content of antioxidant compounds (Castro, 2016). The traditional vegetative propagation of blueberry is not very successful and is considerably limited by seasonal growth (Ostrolucká et al., 2002) and the high costs for large-scale production. Currently, the use of liquid media based on temporary immersion technology in bioreactor, allows a higher growth rate due to the greater contact surface of the explant with the medium and the lower diffusion gradients between the medium and the explant, which facilitates the absorption of nutrients (George 1993). In the present work different methods of disinfection of explants from field and greenhouse of *V. corymbosum* were evaluated, in which the washing times in sodium hypochlorite solution were varied (0, 10, 15, 20, 25 and 30 min.) and the concentration of the same (10, 15, 20 and 25%), having as best result the time from 5 min to 20%, which resulted in 90-100% disinfection. Different combinations of auxins and cytokinins were analyzed. With the combination of cytokinin and auxin at 1 and 0.3 mg / L, respectively, 2 shoots / explant and 0.5 cm increase in shoot length were obtained at 2 weeks. The conditions of frequency and duration of the immersions of the explants in the culture medium were established, where the best response as a function of growth was presented with a 3-min immersion every 8 h.

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Pyr4, a new selectable auxotrophic genetic marker in *Ustilago maydis*

Alma Delia Vega Jiménez[1]., Fidel Landeros Jaime[1,2]., Edgardo Ulises Esquivel Naranjo[1,2]., José Antonio Cervantes Chávez[1,2].
[1]Laboratorio de Microbiología Molecular, Campus Aeropuerto, Facultad de Ciencias Naturales, Universidad Autónoma De Querétaro. Anillo vial Fray Junípero Serra km 8 s/n, carretera a Chichimequillas, Querétaro, Qro. México. C.P. 76140.[2]Cuerpo Académico De Biotecnología y Sanidad Vegetal. Tel: 4427040160. Email: almavj20@gmail.com.

The fungus *Ustilago maydis* produces the corn smut disease, known as huitlacoche, which is specific to *Zea mays* and teozintle. *U. maydis* shows a dimorphic life cycle, the saprophyte phase develops as haploid cells in the yeast-like form and the pathogenic phase is presented as dikaryotic cells characterized by the mycelium morphology, which is able to penetrate the plant and develop tumors or galls in aerial parts of the plant.

In order to carry out studies at genetic and molecular level, is important the availability of selection markers. The auxotrophic markers are based on the use of organisms with mutations in some genes involved in the synthesis of some amino acids or essential compounds, which must be added exogenously to the culture medium. In such a way, it is possible to include wild-type copies of the related gene in the transformation vectors, to allow the proper selection of the transformant organisms. Nowadays, in *U. maydis* all genetic markers available are based on resistance to antibiotics, among them: hygromicin B, carboxin, bleomycin and nourseothricin. Considering the usefulness of auxotrophic markers, which do not suppose any risk of horizontal transfer involving resistance genes, we propose the implementation of an auxotrophic marker for this fungus.

We study the biosynthesis of uracil, in which six enzymes are involved. Dihydroorotate dehydrogenase *(Pyr4),* catalyzes the conversion of dihydroorotate to orotate. Deletion of *this gene* resulted in auxotrophy to uracil as expected, and highly reduced pathogenicity as well. This could be related to the fact that □*pyr4* strains are very sensitive to oxidative and osmotic stress.

We designed pairs of primers to amplify different versions of the *Pyr4* wild-type gene. With the 4042 bp version, we were able to recover transformants prototrophic to uracil and sensitivity to hygromycin B, showing in this way gene complementation. These strains, recovered its capacity to resist osmotic and oxidative stress similar to wild-type strains.

Regarding the functionality of the amplified PCR fragment as a selective marker, pathogenicity test in plants, revealed the recessive nature of the mutation, since when plants were infected with a cross of wild-type strain x *Δpyr4* strain, disease symptoms were observed, similar to those produced by a wild-type cross and the same was observed when a cross of complemented strains were injected in the plant.

Interaction studies at the molecular level protein-peptide, using calmodulin as a molecular target

Isabel Velázquez-López[1], Erika León-Cruz[1], and Martin González-Andrade[1]
[1] Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad Universitaria. Ciudad Universitaria, 04510, Ciudad de México, México. Phone number: +52 5556232254. E-mail: martin@bq.unam.mx

Abstract

The present work contemplates experimental and theoretical studies of protein-peptide interactions, using the calmodulin protein (CaM) as a molecular target. The experimental studies will be carried out using a fluorescent biosensor of the CaM (hCaM-M124C-mBBr), developed by our working group previously; and the theoretical studies through various molecular modeling tools (modeling by homology, docking, molecular dynamics, etc.) using the super computing infrastructure of the DGTIC. This will allow the rational design of new peptides of the CaM protein to be considered prototypes of biopharmaceuticals. Since this protein is considered a molecular target, it is associated with around 300 proteins of pharmacological interest, related to different pathologies such as smooth musculature, behavior and cancer, among the most important. The studies contemplate competition assay between CaM-protein-peptide complexes and CaM-protein-drugs-peptides to establish relationships that lead us to propose more specific bioactive molecules as inhibitors of the CaM protein. The results of this research can be translated into a possible technology transfer or patenting.
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A fluorescent assay for the detection of alkyl glucosides and other non-ionic surfactants

Wendy Xolalpa Villanueva, Gloria Saab Rincón

Departamento de Ingeniería Celular y Biocatálisis, Instituto de Biotecnología, Universidad Nacional Autónoma de México.
Av. Universidad #2001, Col. Chamilpa C.P. 62210, Cuernavaca, Morelos, México.
Tel. +52 (777) 329-1605. gsaab@ibt.unam.mx

Surfactants are amphiphilic molecules able to reduce the surface tension of a solution; they mainly contain a hydrophilic and a hydrophobic region. Usually, the hydrophobic region stands a long aliphatic hydrocarbon chain, whereas the hydrophilic portion comprises of an ionic or non-ionic polar group. Alkyl glucosides (AG) are a group of low-toxic, non-ionic surfactants widely used in the industry with applications in cosmetics, pharmaceuticals, detergents etc. The enzymatic synthesis of AG is a friendly environmental process in contrast to the chemical synthesis where many steps and toxic compounds are required. Directed evolution is a powerful technique to change specificity and improve some properties of enzymes such as stability, efficiency, thermotolerance, etc. However, a bottleneck is to have a suitable activity assay to screen thousands of variants. In this specific case, we need to detect the produced surfactants over the more abundant hydrolysis products. Some analytical methods for AG detection, as HPLC, capillary electrophoresis or gas chromatography, among others, are expensive, time consuming and inconvenient for a high throughput format. Thus, in order to look for a faster and easier way to detect AG we designed a technique based on a previously reported method to determine the critical micellar concentration (CMC) of some non-ionic surfactants. Samples coming from enzymatic reactions are tested in 96-well black plates where the increment in AG concentration takes advantage of the increment in fluorescence of 8-Anilinonaphthalene-1-sulfonic acid (ANS) when it binds to hydrophobic regions. The assay adapted to a multi-well plate allows in such a way several samples can be measured at the same time; it is useful for AG and other non-ionic surfactants as Triton X-100. Standardization of the assay has been carried out, showing a linear response between the fluorescence and the AG concentration.

Influence of bacteria in the digestive system of the worm *Eisenia Foetida* in the degradation of polychlorinated biphenyls

IBQ. Adalberto Zenteno Rojas* Reiner Rincón Rosales, Rocío Meza Gordillo, Victor M Ruiz Valdiviezo, Juan J Villalobos Maldonado.
Instituto Tecnológico de Tuxtla-Gutiérrez, Carr. Panamericana km. 1080, CP 29050, Tel: +52 961 615 0461, Tuxtla-Gutiérrez, Chiapas.
adzer.89@hotmail.com

Introduction. Polychlorinated biphenyls (Bpc´s) are persistent organic chemical compounds. They are classified as recalcitrant xenobiotics and are found within compounds that in greater proportion contaminate the environment and human health. It is estimated that from 1930 to 80 ´ s were produced approx. 1, 200, 000 ton of Bpc´s in the form of complex mixtures, of which 800, 000 tons are found in electrical equipment and in places where they were applied, and others are scattered in the environment. Because of the complexity of its degradation, catalytic biological processes continue to be sought as remediation alternatives. The use of bacteria with degradation potential of toxic chemical compounds is now an important alternative. The objective of this research was to isolate and characterize endosymbiotic bacteria of the digestive system of the worm *E. Foetida*, evaluating the potential of degradation of the decaclorobifenilo.

Materials and methods. Removal Kinetics. The experimental units contained thirty grams of substrate composed of peat moss and excreta of rabbit in the proportion of 85/15 (w/w%) respectively. They were prepared in flasks of 500ml. The total substrate was mixed and adjusted to 75% moisture with distilled water. The experimental units were sterilized following the methodology described by Contreras-Ramos *et al*. (2009) sterilizing at 15 lb for 30 min, three consecutive days. For the contamination of the system was added specific volumes of a solution Decaclorobifenilo concentrated to 1000ppm to adjust to 1000 and 1500 ppm of which were inoculated 30 units per concentration. Each experimental sample was inoculated with adult worms, 3 of them per unit and recording the average weight of the set of worms, the difference between each worm per set was ≤ 0.1 g. The flasks were covered with cloth stoppers to avoid contamination of the environment towards the experimental units. The flasks were incubated at 25 °c and the observation period of earthworm behavior was 72 days. The humidity of the system was controlled by adjusting every 20 days to 75% with distilled water. Isolation of endosymbiotic bacteria from Eisenia foetida. Three soil worms and four enriched culture media were used for the isolation of aerobic bacteria from the earthworm intestine, Congo red agar, crorazón brain infusion agar, nourishing agar and tripticaseína soya agar, inoculated with the material Organic or intestine of the earthworm, at Dilutions 10-05 to 10-08. The extraction of genomic DNA from the pure strains was carried out with the commercial Kit Zymo Research ®. Study of the genetic diversity of endosymbiotic bacteria of *Eisenia foetida*. The genetic diversity of endosymbiotic bacteria in the intestine of the earthworm was carried out using genomic traces, ERIC_PCR (Frans J, 1992), ARDRA and by phylogenetic Gen 16s rRNA (Kit Boye et al 1999). Statistical data analysis. The data obtained were analyzed by analysis of variance with ($p<0.05$).

Results. Final concentrations of decaclorobifenilo in the substrate and earthworm. In this system of elimination of Decaclorobifenilo by means of vermicomposting made by the earthworm *E. Foetida* It has been found that the maximum elimination of Decaclorobifenilo was 95.38% ($p<0.05$) quantified in the first 7 days. On the other hand the bioaccumulation of Decaclorobifenilo by the Earthworm did not present significant statistical difference compared to day 7 and 72, obtaining 26% ($p<0.05$) of bioaccumulation in the Matrix of Worms. Genetic diversity of associated bacteria in decaclorobifenilo degradation. Bacterial identification was performed on days 0, 7 and 72 according to the previously evaluated removal kinetics. 74 bacterial strains were isolated from the intestine of 3 earthworms adapted to the system previously evaluated, 24 of nutritive Agar, 20 of infusion agar brain heart, 18 of soya agar tripticaseína and 12 of red Congo agar. Through the study phylogenetic ERIC_PCR and ARDRA these strains were grouped into 5 phylogenetic groups. By amplification of the gen 16s rRNA of strains type representatives of these phylogenetic groups and the sequencing of the same were found that the 5 groups were composed of bacteria of the gene: *Bacillus, Caryophanon, Paenibacillus* and *Pseudomonas*. According to the Shannon-Weaver index, High bacterial diversity (H = 3.40) and abundance (d = 3.15) were observed over time (0 days). Also on day 7, the kind identified were *Acinetobacter, Bacillus, Klebsiella* and *Staphilococcus*. In this case, the diversity index was H = 3.42 and Abundance D = 3.15. With respect to day 72, the genera identified were *Staphylococcus, Bacillus, Klebsiella* and *Enterobacter* and in this case the diversity index was H = 3.12 and Abundance D = 3.45 said bacterial species has been responsible for soil remediation processes Conntaminados with recalcitrant chemical compounds such as BPC´s (Pieper and Seger, 2008).

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Incorporation of ORF2 from Porcine Circovirus Type 2 into genetically encoded particles by a self-aggregating peptide and its use as subunit vaccine

Jesús Zepeda-Cervantes[1,2], Adolfo Cruz-Reséndiz[1], Alicia Sampieri[1] and Luis Vaca[1]
[1]Instituto de Fisiología Celular, Universidad Nacional Autónoma de México (UNAM), Ciudad Universitaria, Ciudad de México 04510, México.
[2]Programa de Doctorado en Ciencias Biomédicas. UNAM, Ciudad Universitaria, Ciudad de México 04510, México.

Corresponding: lvaca@ifc.unam.mx

ABSTRACT

Porcine Circovirus Type 2 (PCV2) is one of the most important pathogens in pigs around the world. The virus capsid is formed by a single protein known as ORF2. The aim of this study was to evaluate the antigenicity and immunogenicity of protein nanoparticles containing ORF2 from PCV2 fused to the first 110 amino acids of the N-terminus of polyhedrin from the insect virus *Autographa californica nucleopolyhedrovirus* (PH(1-110)). Our group has previously described that polyhedrin is a self-aggregating protein responsible for forming the occlusion bodies from baculovirus. We identified the self-aggregating signal within the first 110 amino acids from polyhedrin. The fusion of proteins to PH(1-110) results in the formation of nanoparticles which incorporates the protein of interest within the particle. Using this system we produced nanoparticles containing PH(1-110) fused to ORF2 (PH(1-110)PCV2). We synthesized these nanoparticles and purify them to immunize pigs to evaluate the immune response generated with these nanoparticles. Pigs immunized with PH(1-110)PCV2 nanoparticles produced antibodies against ORF2 from PCV2 as indicated by western blot and ELISA. Antibodies obtained with PH(1-110)PCV2 nanoparticles were comparable to those obtained using a commercial PCV2 vaccine. These results together suggest that the self-aggregating peptide PH(1-110) can be used for the synthesis of a subunit vaccine against PCV2.

"Identification and characterization of the chitinases and glucanases produced by Wickerhamomyces anomalus and Trichoderma harzianum against crops phytopathogens"

Zepeda Giraud Luis Fernando, Martínez López Cinthya, Araiza Villanueva Minerva Georgina, Hernandez Morfin Katia Daniela, Olicón Hernandez Dario, Guerra Sánchez María Guadalupe.
Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional. Prolongación de Carpio y Plan de Ayala s/n, Col. Santo Tómas, C.P 11340, Delegación Miguel Hidalgo, Ciudad de México. Tel. 57296000 ext. 62339.
Correspondence e-mail: lupegs@hotmail.com

The use of antifungal chemical compounds to control the diseases on agricultural products caused by different phytopathogens, its generating a growing concern because the potential environment impact, appearance of resistant strains and potential damage to human health. All of these have driven the search for new alternatives to the chemical fungicides. One of these alternatives is the use of biocontrol agents that use different mechanisms to control these phytopathogens. However, a new approximation is the use of secondary metabolites that are generated by the antagonist-pathogen interactions. For this work, our goal is to identify and characterize the chitinases and glucanases produced by *Wickerhamomyces anomalus* and *Trichoderma harzianum* during the interaction with the phytopathogens *Colletotrichum gloeosporioides* and *Thielaviopsis paradoxa*. We proved the inhibition capacity of both antagonists against the phytopathogens in solid media. In liquid media we tested the capacity of a filtered supernatant containing the hydrolytic enzymes and were effective against the spore germination for both phytopathogens, as well to the growth of the filamentous form. Also, we observed an increase of the hydrolytic activities of both enzymes when the inductor was dead mycelia, compared to colloidal chitin its used.

Protein dosage of the *lldPRD* operon depends on processing of the primary transcript.

Lidia Esmeralda Angel Lerma, Enrique Merino Pérez, Roberto Coria Ortega, Adrián Fernando Álvarez, Dimitris Georgellis.
Departamento de Genética Molecular, Instituto de Fisiología Celular. Universidad Nacional Autónoma de México, 04510 Ciudad de México, México. 1 esme.angel.lerma@gmail.com Phone: (52) 55 56225738

The ability of *Escherichia coli* to grow on L-lactate as a sole carbon source depends on the expression of *lldP*, whose product, LldP transports L-lactate across the membrane, and *lldD*, whose product, LldD, oxidizes L-lactate to the central metabolite pyruvate. These genes are on the lldPRD operon, which in addition contains the *lldR* gen, a dual transcriptional regulator.
An interesting feature of this operon is that the stop codon of the upstream gene overlaps the start codon of the downstream gene in such a way that the three coding regions are arranged in three different reading frames. Also, it is curious that the cistron encoding a transcriptional regulator (*R*) is located between the permease (*P*) and the dehydrogenase (*D*) encoding genes. This genetic organization portrays a paradox as the regulator protein is expected to be required in relatively small amounts whereas the two enzyme-encoding genes are required in much higher amounts. In this study we report that protein dosage of the *lldPRD* operon is not modulated on the transcriptional or translational level. Instead, modulation of protein dosage is attributed to RNAse E-dependent mRNA processing events that take place within the *lldR* mRNA, leading to the immediate inactivation of *lldR*, and to differential segmental stabilities of the resulting cleavage products. Thus, RNAse E-dependent mRNA processing ensure the fine-tuning of the expression of the individual genes of the *lldPRD* operon, and thereby provide a notable variation of the previously documented examples of gene expression

Transcriptomic landscape of the radioresistance in breast cancer

Elena Aréchaga Ocampo[1†*], Isidro Xavier Pérez Añorve[2†], Claudia Haydée González de la Rosa[1], Ernesto Soto Reyes[1], Fredy Omar Beltrán Anaya[3], Oscar del Moral Hernández[4], Villegas-Sepúlveda Nicolás[5].
[1]Department of Natural Science, Metropolitan Autonomous University, Cuajimalpa Unit; [2]Natural Sciences and Engineering Postgraduate, Metropolitan Autonomous University, Cuajimalpa Unit; National Institute of Genomic Medicine[3]; Autonomous University of Guerrero4, CINVESTAV-IPN[5].
UAM-C. Av. Vasco de Quiroga 4871, Cuajimalpa de Morelos, Santa Fe Cuajimalpa, Postal Code: 05348, Mexico City. †Equal contribution; *earechaga@correo.cua.uam.mx

Breast cancer is one of the most incident and mortality tumors worldwide. Radioresistance is frequently develops in breast tumors, which is related to worse prognosis. Here we developed a radioresistance model of luminal and triple negative breast cancer tumors (MCF-7RR and MDA-MB-231RR) to identify radiation resistance gene signature. Using clonogenic survival assays, we demonstrate the greater surviving fraction after radiation of the radioresistant cells compared to parental cells. To correlate radioresistance to gene expression we analyzed the transcriptoma of MCF-7RR and MDA-MB-231RR cells by microarrays (Affymetrix 2.1). We found a set of 152 genes modulated in luminal breast cancer cell model MCF-7RR, while in triple negative breast cancer cell model MDA-MB-231RR were identified 158 genes. Our analysis by functional protein association networks, gene ontology and biological pathways showed that gene signature in MCF-7RR were associated to communication and adhesion cellular, control transcripcional and signaling transduction. By the other side, in MDAMB-231RR gene signature was related to transcription regulation, DNA repair, DNA replication and chromosomal stability by component of chromatin accessibility, besides there are important components of the immune response and inflammatory pathways.Our results identify distinctive molecular processes for resistance to radiotherapy in luminal and triple negative tumor cells respectively. These data open new insights into identification of possible biomarkers for breast cancer.

Advances in the molecular study of the musculoskeletal system in tropical gar (*Atractosteus tropicus*).

José Natividad Arias Jiménez; Talhia Martínez Burguete; Simrith Elizabeth Córdova de la Cruz; Carlos Alfonso Álvarez González & Missael de Jesús Salcedo Hernández.
Universidad Juárez Autónoma de Tabasco. Carretera Villahermosa-Cárdenas Km. 0.5 S/N, entronque a Bosques de Saloya. Villahermosa, Tabasco. C.P. 86039
jarias@email.ifc.unam.mx; Tel.: (669) 164 7844

The tropical gar (*Atractosteus tropicus*) is an endemic fish belonging to the family *Lepisosteidae* that lives in fluvial bodies of the Southeast Mexico and Central America, playing an important ecological and economic role. There is limited genetic information available for the species, however it occupies an interesting phylogenetic position between tetrapods and teleost fishes, which is why it is currently considered as a new biomedical model.

The development of the musculoskeletal system provides mechanical support to the tissues, enabling locomotion, feeding and body posture. At the molecular level, it is regulated by extracellular signaling molecules and intracellular transcription factors.
In this project we have investigated the genes involved in myogenesis and osteogenesis, finding a high similarity of sequences of up to 70% with some groups of vertebrates, demonstrating the evolutionary divergence of the members of the family *Lepisosteidae* and of other "ancient fishes" that did not suffer the duplication of their genome. The relative expression of these genes concurs with the stages of the indirect development that the species undergoes during its larval phase until before reaching its juvenile stage.

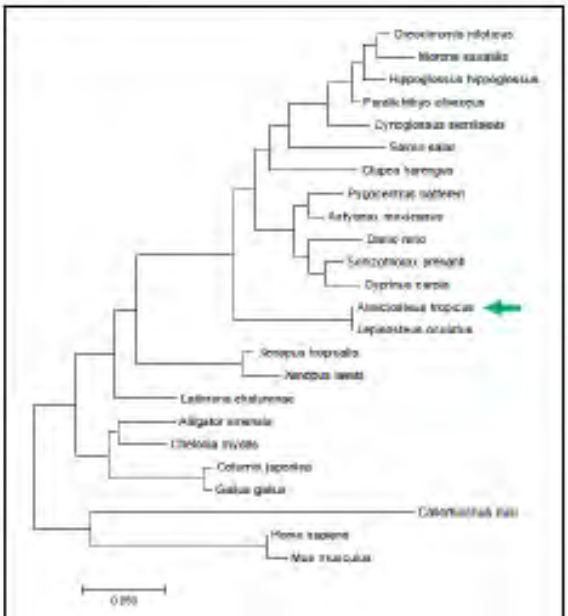

Figure 1.- Phylogenetic analysis of *A. tropicus* myogenin amino acid sequence relative to myogenin from other vertebrates.

The transcriptome of this species was generated from organisms of different stages of development allowing to analyze globally the gene expression and identify the regulatory elements that they share with humans, providing a new biomedical model that is comparable to human biology.

Chia seeds extract modify the expression of *Nos3* and *Bdkrb2* genes in hypertensive rats

Gerardo Ismael Arredondo-Mendoza, Maricela Villarreal-García, Francisco Javier Guzmán-de la Garza, Blanca Edelia González-Martínez, Eduardo Campos-Góngora.
Universidad Autónoma de Nuevo León, Centro de Investigación en Nutrición y Salud Pública. Ave. Dr. Eduardo Aguirre Pequeño S/N, Mitras Centro, 64460 Monterrey, N.L. Phone: (81) 1340 4890 Ext: 3062. Email: gerardogiam@hotmail.com

Introduction: High Blood Pressure (HBP) is the principal risk factor to develop cardiovascular disease. HBP is regulated by the renin-angiotensin system, particularly the angiotensin-converting enzyme (ACE). ACE inhibitors are used as first-line therapy, however, these produce secondary reactions. Searching of natural source is an alternative for HBP treatment and salvia species have shown a hypotensive effect. We analyzed the effect of an ethanolic extract of chia (*Salvia hispanica* L.) seeds (EESH) on the expression of *Nos3* and *Bdkrb2* genes, involved in HBP regulation.

Methods: A model of rats with induced hypertension was generated by the administration of NG-nitro-L-arginine methyl ester (L-NAME), nitric oxide synthesis inhibitor. Hypertensive rats (12) were allocated to three different treatments; the first group received captopril, the second group received EESH and the last group remained with the administration of L-NAME. Control group (healthy group, 4 rats) was treated with distilled water during the whole study. In the second phase, the gene expression analysis was realized by RT-PCR in samples of cardiac tissue, using specific oligonucleotides designed for *Nos3*, *Bdkrb2* and *Gapdh* genes; the comparisons of level expression between the groups was performed using statistical analyses with ANOVA test.

Results: After treatments, rats that were receiving L-NAME showed an increment on blood pressure levels (31.2%). The administration of captopril and EESH decreased 0.2% of blood pressure levels. Gene expression analysis showed that the administration of L-NAME decreased significantly the expression of *Nos3* gene, in comparison to basal levels (healthy rats). In the hypertensive rats treated with captopril there were no changes in the expression of *Nos3*. The administration of EESH produced a similar effect as captopril in *Nos3* expression levels. The *Bdkrb2* gene expression did not show significative changes in the different treatment groups.

Conclusion: The EESH have a similar effect to the captopril diminishing the blood pressure of hypertensive rats. Our results suggest that this effect is through a mechanism that involved the transcriptional regulation of *Nos3* gene.

Minigenes as reporter systems for functional genomics.

Elizabeth Cavazos Benhumea, Victor Alejandro García Fidencio, Alejandro Bustos Cortez,Luis Enrique Jiménez Hernández Carlos Fabián Flores Jasso and Selma Eréndira Avendaño Vázquez.

Instituto Nacional de Medicina Genómica, INMEGEN, Periférico Sur 4809, CDMX 14610,México. Tel. 5553501900. email: seavendano@inmegen.gob.mx

With the contribution of initiatives like *Human Genome Project* (concluded in 2003), the *International HapMap Project* (concluded in 2005) and *The 1000 Genomes Project* (concluded in 2015), we now have a set of research tools that make possible to find the genetic contributions to complex traits and common diseases, as well as many key insights into the human population history and evolution. These tools include computerized databases that contain the reference human genome sequence, a map of human genetic variation and a set of bioinformatics technologies that can quickly analyze whole-genome data.

The genome-wide association study (GWAS) is an approach that involves rapidly scanning

markers across the complete sets of DNA, or genomes, of many people to find genetic variations associated with a particular trait, being normal or pathological one. In the case of pathological traits the goal is that once new genetic associations are identified, the information can be use to develop better strategies to detect, treat and prevent the disease. Although GWAS have the potential to point as responsible or epistatic contributors to many genes, what is really missing for to develop effective treatments is the knowledge of how Most of the gene variants associated with a pathogenic disease do not alter reading frames in a way that leads to truncated proteins or annul active sites. We can suspect that the great majority of gene variants would affect processes of gene expression regulation.

It is estimated that 90% of the human protein coding polymerase II transcripts is alternatively spliced and an increasing number of human diseases arise from aberrant splice process. So, these pathogenic genetic variants could influence in the splicing process but this requires experimental verification.

Our main interest is to essay how genetic variants associated with diseases affect at splicing process and consequently alter gene function and cause disease. To test this, we designed the bioinformatic tools that allow to identify the genetic variants associated with disease that might affect alternative splicing regulation, and we use minigenes transfected into cells, a common technique used to analyze the regulation of an alternative splicing, to test the pathological genetic variants molecular mechanism.

As a prof of concept, we generated minigenes as reporter system to study the complex traits related to addiction and insulin resistance. The first minigene contains the coding sequence of dopamine D2 receptor caring an siRNAs resistant sequence and containing the complete introns 5 and intron 6 sequences. The second minigen had the sequencing of the insulin receptor with the complete intron 10 and a short version of the intron 11 to test the change of splicing of INRA and INRB.

Study of Mitochondrial DNA in Modern Nahuas from Central Mexico: Overview of the Genetic Relationships.

Víctor Hugo Avilés Chávez[1], Aurora Millán Sierra[1], Héctor Alessandro López Hernández[1], Ernesto Garfias Morales[1], Angélica González Oliver[1].

[1]Departamento de Biología Celular, Facultad de Ciencias, Universidad Nacional Autónoma de México. Ciudad de México. C.P. 04510.
Autor responsable: Angélica González Oliver. Departamento de Biología Celular, Facultad de Ciencias, Universidad Nacional Autónoma de México. Ciudad de México. C.P. 04510. Teléfono: 5622-4910. Correo electrónico: goliver@unam.mx

The Nahua group is the most numerous in Mexico, they conserve and practice their own cultural traditions, belong to the Yuto-Aztec linguistic family and live in Central Mexico.

The study of mitochondrial DNA (mtDNA) has shown that nearly all-American Natives present one of the five founding haplogroups of Asian origin called A, B, C, D, and X, and exhibit at least 15 haplotypes of which A2, B2, C1, D1 and X2 are the most common.

In this study we identified the frequencies of mtDNA haplogroups in modern Nahua individuals, unrelated to each other, who live in the states of Hidalgo, Puebla and San Luis Potosí. The results obtained were compared to those reported for other modern and ancient Nahua populations in order to understand the genetic relationships.

Haplogroup A was the most frequent with frequency values in the range of 46% to 74%. The haplogroup D was the least frequent with low frequencies from 2% to 17%. The modern and ancient Nahua populations do not show a clear distribution pattern of the mitochondrial haplogroups four frequencies, as has been observed for other populations of Mesoamerican origin. The results show significant genetic differences between the Nahua populations, most notably comparing the modern Nahuas of Veracruz with those of Central Mexico; this could be either to the regional history of the populations or their origin.

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Effect of epigenetic drugs on regulation of *c-MYC* promoter in hepatic cell lines.

Paulina Janeth Barraza Reyna, Jorge Eduardo Vargas Gómez, Alain Jesús Aguirre Vázquez, Diana Reséndez Pérez, Mario Abelardo Bermúdez de León
Centro de Investigación Biomédica del Noreste del Instituto Mexicano del Seguro Social. Dirección: Calle 2 de abril 501, Col. Independencia, Monterrey N.L. C.P. 64720. Teléfono. 01 (81)8190-4036. Correo electrónico: distro71@yahoo.com

Hepatocarcinoma is one of the most aggressive types of cancer, being the second cause of cancer death worldwide. Among its common genetic alterations it has been found an overexpression of *c-MYC* oncogene. Other causes of hepatocarcinoma are associated with hepatitis B or C virus infection, and alcohol consumption. In murine models it has been demonstrated that conditional overexpression of *c-MYC* gene can induce liver cancer and its inactivation induces a regression of tumor growth. The development of cancer is not only due to mutations in the DNA sequence, also by epigenetic modifications that alter the regulation of genes, such as hypomethylation of genomic DNA, which was the first modification described in neoplastic cells. *c-MYC* gene has four promoters, being P1 and P2 responsible for generating the highest amount of transcript, 10-25% and 75-90%,respectively. The activity of the promoters can be affected by epigenetic modifications, therefore the use of chromatin modifying molecules could be an important tool to modify the regulation of the *c-MYC* gene in cancer cells. In this project we propose to evaluate the activity of P1 and P2 promoters in response to epigenetic drugs in hepatic cellular models HepG2 and HuH7. Drugs that will be used in this project (called F5 and F6) belong to the basic medicine scheme of health sector and have been shown a highly significant effect on the repression of c-MYC in HepG2 cells.

Objective. To evaluate the activity of *c-MYC* promoter in hepatic cell lines HepG2 and HuH7 by two epigenetic drugs.

Methodology. Constructs pGL3-MYC-P1 pGL3-MYC-P2 and PGL3-MYC-P1-P2, harboring

P1, P2 and P1-P2 c-MYC promoter, respectively, were characterized by enzymatic digestionand sequencing. The co-transfection of the constructs and phRL-CMV vector (to normalize the transfection efficiency) was carried out in HepG2 and HuH-7 cells using polycationic lipids. After 24 h post-transfection, the cells were treated with F5 and F6 epigenetic drugs.Cells transfected were lysed and the luciferase activity assays were performed using the Dual-Luciferase system.

Conclusions. Luciferase activity of constructs pGL3-MYC-P1, pGL3-MYC-P2 and pGL3-MYC-P1-P2, harboring the promoter of c-MYC, was determined in hepatic cells, where pGL3-MYC-P1-P2 showed the higher activity.

"Impact of high fat diet on the intranuclear location of the circadian gene *Dbp*"

Fernando Becerril Pérez, Ignacio Pacheco Bernal, Lorena Aguilar Arnal*
Instituto de Investigaciones Biomédicas, UNAM
* Instituto de Investigaciones Biomédicas, Sede Tercer Circuito Escolar, Apartado Postal 70228, Ciudad Universitaria 04510, México, CDMX. Office 562-28938.
loreaguilararnal@iibiomedicas.unam.mx

Introduction: The molecular architecture of the circadian clock consists of transcriptional/translational feedback loops of 24 hours length. The core clock component is composed of a heterodimeric transcription factor (CLOCK/BMAL1) that binds its DNA recognition motif (E-box) and drives the expression of the Clock Controlled Genes (CCG). Among these CCG, the circadian repressors *Per* and *Cry* are transcribed. This whole loop has an approximate timing of 24 hours (1). Another CCG is *Dbp*, a PAR bZIP transcription factor involved in circadian xenobiotic detoxification through rhythmic transcription of genes involved in this process.
Circadian gene regulation has been studied at different levels: transcript availability, chromatin accessibility, chromatin recruitment of clock proteins and recently, chromatin architecture. The latter describes the location and architecture (interactions) of genes or regulatory elements inside of the nucleus (2). These interactions have functional implications in gene expression.
Recently, it was shown that circadian genes tend to rhythmically colocalize with other circadian genes or elements according to their transcriptional state. Such interactions were shown to be dependent on BMAL1 (3).
Aim of the study: It has been described that different diets can reprogram the hepatic circadian transcriptome. Hence, the aim of this study is to determine the impact of high fat diet feeding in the circadian interactome of the circadian gene *Dbp* in livers of mice fed with normal chow or high fat diet (HFD).
Method and results: C57BL/6 mice were fed either normal chow or HFD during 12 weeks. HFD fed mice showed a metabolic phenotype, with increased blood glucose and impaired glucose and insulin tolerance. Animals were sacrificed at ZT 6 and ZT18 (ZT, zeitgeber time; with lights-on at ZT0) and tissues were recovered. *Dbp* expression was measured in the liver by qPCR, finding significative differences between normal chow and HFD fed mice. The spatial architecture of the *Dbp* locus has been analyzed using Chromosome Conformation Capture (4C-seq).

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HIF-1 and VEGF gene expression by valproate in human hepatome cells

Mario Bermúdez de León[1,2], Edgar X. Franco Juárez[2], María Elena Camacho Moll[1,2], María Guadalupe Moreno-Treviño[1]

[1]Departamento de Ciencias Básicas, Vicerrectoría de Ciencias de la Salud, Universidad de Monterrey. 2Laboratorio de Biología Molecular, Centro de Investigación Biomédica del Noreste, Instituto Mexicano del Seguro Social.
Calle 2 de abril 501, Col. Independencia, Monterrey, Nuevo León.
Teléfono (81)8190-4035. Correo-E: distro71@yahoo.com.mx

In 2015, liver cancer was the second cause of death worldwide with 788,000 deaths (INEGI, 2018). In Mexico, in the period 2004-2006, the incidence of liver cancer increased by 60% and mortality by 14%. The morbidity-mortality ratio in the case of liver cancer is close to 1, which means that the survival rate of those patients diagnosed with this disease does not exceed 5 years. Among the main causes of this type of cancer, in addition to the genetic and epigenetic components, are viral infections, liver disease and cryptogenic cirrhosis. The cancer cells are characterized by sustained proliferation, evasion of cell tumors and apoptosis signals, and by the mechanisms activated in the cascade called invasion-metastasis that finally induce angiogenesis. The intention of this project is based on the expression of the HIF-1 gene, which encodes for HIF-1α induced in hypoxic conditions in the cancer process, which is the transcriptional activator of the VEGF gene. VEGF is a protein responsible for the development of blood vessels and intrinsically involved in tumor angiogenesis. As an experimental approach, a treatment based in valproic acid, as a chromatin-remodeling drug able to inhibit the histone deacetylases, in cultured cells. It is known that histone deacetylases are altered in cancer cells and their inhibition is one of the strategies that have been suggested for cancer control. In the present study, HepG2 cells, derived from a human hepatoma, exposed to a cobalt chloride, as a hypoxia inducer, and treated with valproic acid have been used. The gene expression analysis for HIF-1 and VEGF has been carried out by RT-qPCR using TaqMan® probes. The results demonstrated that cobalt chloride, but not valproate, is able to inhibit HIF-1 and VEGF gene expression.

Metabolic regulation of β-NAC subunits in the yeast *Saccharomyces cerevisiae*

José Ernesto Bravo Arévalo[1], María Clara Avendaño Monsalve, Soledad Funes
Departamento de Genética Molecular, Instituto de Fisiología Celular, UNAM, C.P. 04510, CDMX.
[1]e-mail and phone number: joser7190@gmail.com, +525556225679

The Nascent-polypeptide Associated Complex (NAC) is a heterodimeric cytosolic chaperone conformed by an a and b subunits that binds to the ribosome exit tunnel and engages the first 20 amino acids of the nascent polypeptide. Its activity was associated to prevent non-specific folding as the protein emerges from the ribosome.
In the yeast *Saccharomyces cerevisiae,* the NAC complex possess two β paralogs (encoded by the *EGD1* and *BTT1* genes) that arose from whole genome duplication.Additionally, each paralog has different function since Egd1 subunit (β1-NAC) attach to most nascent cytosolic proteins and cargo destined to Endoplasmic Reticulum and, in contrast, Btt1 subunit (β2-NAC) was reported to participate in the cotranslationally model of mitochondrial protein import process. Nonetheless, little is known about the regulation of expression of the paralogs and how its expression affects the cellular fitness. We assess the regulation of the b1- and β2-NAC subunits by constructing a set of mutants whose genomic loci were exchanged between the *EGD1* and *BTT1* genes. Additionally, we used mutants lacking either one of the b paralogs to compare the growth phenotype and evaluate the expression change in different carbon sources and in oxidative stress induction between the different strains. By and large, we encountered that the regulation relies upon carbon source and growth phases more than stress responses associated with oxidative damage.

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Académico (DGAPA-UNAM).

DNA methylation alterations in adipocytes derived from obese diabetic patients

Federico Centeno, Paulina Baca Peynado, Elahe Mirzaeicheshmeh, Ernesto Sánchez, Angélica Martínez, Carlos Zerrweck, Francisco Barajas, Lorena Orozco.

Laboratorio de Inmunogenómica y Enfermedades Metabólicas. Instituto Nacional de Medicina Genómica. Periférico Sur No. 4809, Col. Arenal Tepepan, Delegación Tlalpan, Ciudad de México. C.P. 14610. fcenteno@inmegen.gob.mx. Tel 53501962

BACKGROUND

Obesity is a complex metabolic disease influenced by genetic and environmental factors. Obesity induces a constant state of low-grade inflammation with infiltration and activation of immune cells that increases the production of proinflammatory cytokines, contributing to insulin resistance, type 2 diabetes and cardiovascular disease. Recently it has been reported that epigenetic alterations, especially DNA methylation, may have an important role in the pathogenesis of metabolic diseases and that these alterations may be present in the tissue precursor cells, thus we evaluate the alterations in DNA methylation in adipose tissue from patients with diabetes and in cultures derived from these tissues.

METHODS

22 individuals with BMI ≥ 35 kg/m^2 were recruited with (OBD) and without diabetes (OND). A sample of visceral adipose tissue (VAT) was taken during the surgery process. A portion of tissue was used for nucleic acids extraction and a portion was employed to mesenchymal stromal cells (MSC) isolation. MSC cultures were cultured in DMEM supplemented with 10% FBS and differentiated to mature adipocytes with differentiating media. DNA methylation was evaluated in VAT, MSC and *in vitro* differentiated adipocytes with Infinium Methylation EPIC BeadChips. Gene expression was assessed with Affymetrix Clariom S Human Arrays. R program was used for arrays and statistical analysis.

RESULTS

We identified altered CpG sites in adipose tissue from OBD patients, as well as in the cell cultures derived from the patients. Several of the alterations found in TAV were retained in MSC cultures and some of them persists during the *in vitro* differentiation processes to mature adipocytes. We also found an impact of altered DNA methylation on gene expression.

Characterization of the microRNA-137 in the breast cancer cell line MDA-MB-231

Andrea Viridiana Cervantes Ayala, Ruth Ruiz Esparza-Garrido y Miguel Ángel Velázquez Flores

Laboratorio de Genómica Funcional de la Unidad de Investigación Médica en Genética Humana del Hospital de Pediatría –Silvestre Frenk Freund", Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social (IMSS).

* Autor para correspondencia: Dr. Miguel Ángel Velázquez Flores. Tel: (+52 55) 56276900 ext. 22409. Correo electrónico: dr.velazquez.imss@gmail.com

Breast cancer (BC) is one of the biggest causes of mortality among women. This cancer is a highly heterogeneous group of diseases that show diverse clinical and molecular characteristics. Currently, many efforts have been made to identify possible biomarkers that improve the BC diagnosis and prognosis. BIK (BCL2-Interacting Killer (Apoptosis-Inducing)), a protein that has a role in the apoptosis induction, it has been identified inside the nucleus of MDA-MB-231 (M231) cells and its interaction with DNA, which included the microRNA-137 genomic region. Based on this, we characterized the miR-137 expression and started the identification of BIK function in the cell line M231. The mRNA expression of certain molecular markers for M231 and for both miR-137 and BIK, it was performed by qPCR. Also, prediction of the biological pathways where this miRNA could be involved were identify with the DIANA-miRPath application. The Epithelial markers CDH1 (Cadherin 1) and EPCAM (Epithelial Cell Adhesion Molecule) showed, respectively, a -6 ± 0.3 and -5.7 ± 1.4 fold decrease in their expression in the M231 cells compared to HMEC cells. In contrast, EMT (Epithelial-mesenchymal transition) markers, VIM (Vimentin) and SNAI1 (Snail Family Transcriptional Repressor 1), were overexpressed (14 ± 5.4 and 9 ± 4.8 fold increase) in the M231 cells relative to HMEC. The miR-137 expression was 3 ± 0.02 fold decreased in the M231 cells compared to HMEC. On the other hand, the BIK expression was 0.66 ± 0.13 fold increase in the cells M231 in comparison to HMEC. Predictions showed that validated and predictable targets participated mainly in the MAPK (Mitogen-Activated Protein Kinase) signaling pathway and in signaling pathways regulating pluripotency of stem cells. In conclusion, our data indicated that the M231 cells –used in this study- have the molecular features previously reported for this cell line. In addition, the decrease in the miR-137 expression could be related to the malignancy of the cells M231 by regulating signaling pathways (MAPK and pluripotency) associated with cell cycle regulation. Finally, the involvement of BIK as transcription factor by regulating the miR-137 expression remains to be demonstrated.

Intra- and inter-plasmid regulation of conjugative transfer in *Sinorhizobium fredii* strain GR64

Laura Cervantes-De la Luz[1], Gonzalo Torres-Tejerizo[2], Eunice López-Fuentes[1,3], Fabiola Miranda-Sánchez[1] and Susana Brom-Klanner[1] .
[1]Programa de Ingeniería Genómica, Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México, AP 565-A, Cuernavaca, Morelos, México. [2]Instituto de Biotecnología y Biología Molecular, CCT-La Plata-CONICET, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina.[3]Present address: División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica, San Luis Potosí, México.
lcervant@ccg.unam.mx

Rhizobial strains are interesting because of their ability to form nitrogen fixing nodules in symbiosis with leguminous plants. There is evidence suggesting that conjugative transfer (CT) has impacted on the diversity of rhizobial strains. *Sinorhizobium fredii* GR64 was isolated from bean nodules in Granada, Spain. This strain contains 3 plasmids in addition to the chromosome: pSfr64a (183 Kb), the symbiotic plasmid (pSym) pSfr64b (460 Kb), and megaplasmid pSfr64c (>1000 Kb). pSfr64a and pSfr64b contain a complete set of transfer genes. Also, they contain quorum-sensing (QS) regulatory genes: *traRa - traIa* on pSfr64a, *traRb - traIb* on pSfr64b, and *ngrI - ngrR* on the chromosome.
Although both pSfr64a and the pSym pSfr64b contain a complete set of transfer genes, CT of each plasmid depends on the presence of the other. To elucidate what causes this phenomenon, we analyzed mutants in the regulatory genes of both plasmids, and determined their expression in the wild type and mutant backgrounds, by transcriptional fusions with reporter genes and by qRT-PCR.
The results showed that TraRa, TraIb, TraRb and NgrI are required for transfer of both plasmids. Regarding the expression analysis, the TraIa-TraRa and TraIb-TraRb complexes induce transcription of the pSfr64a transfer genes, while TraIb-TraRb and TraRa induce transcription of the genes on pSfr64b.
We suggest that NgrI forms a complex with TraRa for the induction of the pSym transfer genes. We observed that in a derivative containg only a cloned *traRa* gene (lacking the rest of the plasmid), the pSym can perform CT, but if a mutation is introduced in the chromosomal *ngrI* gene, the strain looses its transfer ability.
Overall, our results indicate that a regulatory circuit is formed between both plasmids, because the expression of the homoserine lactone synthatese encoding TraI gene from each plasmid also requires the TraR transcriptional regulator from the other plasmid.
Additionally, the expression of the transfer genes from both plasmids, depends on chromosomal QS-related regulatory genes.
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Use of CRISPR-Cas9 to edit the promoter of the microRNA effector protein

Diana Chavira Desales, C. Fabián Flores-Jasso
Instituto Nacional de Medicina Genómica
CP. 14610. Phone: +52 (55) 5350-1900 ext. 1138. cfflores@inmegen.gob.mx

The possibility of editing the genome of an organism has provided us the ability to study the function of gene products and their molecular mechanisms. A technology that has revolutionized the life sciences is the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) system since it allows gene editing with high precision and ease. This system is integrated for a CRISPR-associated endonuclease (Cas protein) and a guide RNA (gRNA). The nuclease Cas is guided by the gRNA towards the specific sites in the genome to introduce cleavages. The CRISPR-Cas9 has been utilized to generate knockouts of several genes, point mutations and substitutions for several regions in the genome —including regulatory regions, such as promoters. By using this technology, the genome now is easily edited or modulated in practically any organism.

microRNAs are small RNAs that regulate gene expression by binding directly to the 3□-UTRs of most messenger RNAs. microRNAs exert their function by guiding the Argonaute proteins to their target mRNAs. Despite most research has been done unveiling the function and biogenesis of microRNAs, little is know about their decay or turnover. The aim of this project is to develop a tool that enables to study the decay of Argonaute proteins and microRNAs in living cells. Here we use the CRISPR-Cas9 system to edit the genome of *Drosophila melanogaster* cells, and substitute the promoter of *Argonaute1* by the Tretracycline Response Element (TRE) in order to generate a system by which the transcription of Ago1 is metabolically regulated by the addition of tetracycline analogs. The details of our experimental approach will be discussed at large. This tool will permit to study the turnover of Argonaute proteins and its association with miRNAs.

Functional effect of variants with uncertain clinical significance (VUS) of the BRCA1 / BRCA2 genes in Mexican women

Manuel Misael Coca-González, Jonathan Silva-Garcia, Luis Elias-Brito, Eduardo López-Urrutia[1] y Carlos Pérez-Plasencia[2].
Facultad de Estudios Superiores Iztacala. UNAM. Av. De Los Barrios 1, Hab. Los Reyes Ixtacala Barrio de los Árboles/Barrio de los Héroes, 54090 Tlalnepantla, Méx.
[1]e_urrutia@unam.mx [2]carlos.pplas@gmail.com. Tel: 56231333 Ext.39807

Among all cancer types, breast cancer is the neoplasm with the highest incidence and mortality among between 25 and 60 year-old women in Mexico. About 10% of breast cancer cases start with germline mutations in the BRCA1 and BRCA2 genes, which encode proteins with a key role in DNA damage repair. These mutations can be deleterious, frameshift or generate premature stop codons. However, there are also mutations with uncertain clinical significance –known as variants of unkown significance, VUS– which apparently do not alter the function and structure of the protein, but can possibly affect co-transcriptional processes such as splicing. This process depends on the recognition of sequences by the spliceosome and its auxiliary factors to remove introns and yield a functional mRNA that can be translated to a functional protein. In 2012, our group conducted a study in 39 Mexican patients to identify mutations in BRCA1 and BRCA2 and found 9 VUS, here we analyze their effect on splicing *in silico* and *in vitro*. Bioinformatic analyzes showed that 7 of the 9 mutations have repercussions in the mRNA splicing, potentially ; hence, we constructed minigenes to obtain functional validation of wether these mutations have an aberrant or no effect on splicing.

Methylation profile distinguishes different outcomes in patients with locally advanced cervix cáncer

Carlos Manuel Contreras-Romero[1] Alejandro Zentella-Dehesa[2] and Carlos Pérez-Plasencia[1]
Instituto Nacional de Cancerología, Av. San Fernando No. 22, Col. Sección XVI Delegación Tlalpan, C.P. 14080 México, D.F[1].
Instituto Nacional de Ciencias Médicas y de Nutrición Salvador Zubirán, Unidad de Bioquimica, México, Distrito Federal[2].
Correspondence author: Av. San Fernando No. 22, Col. Sección XVI Delegación Tlalpan, C.P. 14080 México, D.F. carlos.pplas@gmail.com

Multiple studies have shown that epigenetic events play a significant role in the development and progression of cervical cancer. One of the most characteristic changes is the hypermethylation of CpG Islands in promoter regions, this is related to transcriptional repression; On the other hand, the hypomethylation of DNA contributes to the development of cancer, generating chromosomal instability.

Evidence suggests that aberrant DNA methylation is involved in precancerous stages that can progress rapidly and generate dysplasias. Epigenetic changes in neoplastic cells not only provide targets for drug therapy, they also offer unique possibilities for early diagnosis of cancer. Several studies show that methylation can be directly related to the response to therapy, aberrant methylation patterns cause the silencing of key genes for the response to treatment. In this study, a DNA microarray was analyzed where the methylation pattern of two groups of tumor samples from patients with locally advanced cervical cancer was compared, with one group of patients responding to the treatment and the other not responding. The methylation pattern of these two groups was compared and candidate genes were selected to validate their methylation status in a new group of patients with locally advanced stage cervical cancer who responded and did not respond to treatment. A differential pattern has been found so far in the promoter region of the CTU1 gene between both patient sections.

Key words. Epigenetic, methylation, cervical cancer, therapy response.

Characterization of mRNA methylation (m6A) in response to extracellular stimulus.

Perla Diana Coronado Monroy and Selma Eréndira Avendaño Vázquez.

Instituto Nacional de Medicina Genómica, INMEGEN, Periférico Sur 4809, CDMX 14610, México. Tel. 5553501900. email: seavendano@inmegen.gob.mx

In 1970 the methylation of the 6th nitrogen of the adenine, also termed as m6A, was discovered. Nowadays it is regarded as the most broadly and prevalent internal modification in eukaryotic mRNAs and lncRNAs as well (Laptev et al., 2015; N. Liu & Pan, 2016).

The m6A modification is set in place, removed or recognized by a broad group of proteins known as *writers*, *erasers*, and *readers* respectively. The multiproteic complex formed by: METTL3, METTL14, and WATP is responsible of setting the m6A mark on the adenosine (Jianzhao Liu et al., 2014); whereas two proteins are in charge of the demethylation process, FTO and ALKBH5 (Jia et al., 2011) (Zheng et al., 2013). Due to the last two addressed proteins, is that the m6A modification have been regarded as a dynamic process.

Due to methods like MeRIP-seq (Dominissini et al., 2012), or miCLIP (Bastian Linder, et al., 2015), it has been possible to identified the existence of different cell-type-specific m6A-RNA methylation patterns (Saletore et al., 2012), which suggests that the m6A modification may have different biological roles.

With the studies published so far, it is possible to propose that the m6A modification of the mRNA is a dynamic process of posttranscriptional regulation, which endows the cell with the capability to adapt to changes in its microenvironment.

It has been suggested that FTO does have a participation in the control of energetic homeostasis (Scheid et al., 2014; Speakman, Rance, & Johnstone, 2008), regarded as the control between the energy expenditure and energy intake overtime (Schwartz, Woods, Jr, Seeley, & Baskin, 2000).

The participation of FTO in the control of energetic homeostasis has been suggested not only due to its association with fat mass and obesity, but also because FTO is overexpressed in cerebral regions that control food intake and energy expenditure as the hypothalamus (McTaggart et al., 2011). Also, it has been reported that SNP in FTO, such as rs9939609, impacts directly the circulating levels of PYY and/or acyl-ghrelin neuropeptides; these last are part of the coordinated orexigenic and anorexigenic signalling system, which controls the energetic homeostasis regulating the food intake and energy expenditure.

Orexigenic signals works stimulating the energetic intake and reducing the energetic expenditure; whereas, anorexigenic signals inhibits the energetic intake and promotes the energetic expenditure. That means, clearly opposite effects.

Given that the cells receive constant extracellular signals, as chemical messengers present in its microenvironment, these signals can be part of the mechanisms which coordinates the activity of writers and readers proteins, leading to different patterns of mRNA methylation.

In order to explore this, we are using the orexigenic and anorexigenic signalling system to determine: 1) whether m6A methylation in RNA is driven by extracellular signals.; 2) if different extracellular signals induce specific m6A-RNA methylation patterns; and 3) if the m6A patterns in the RNA are exchanged, as an adaptive form to extracellular stimuli.

mir- 26a, epigenetic regulator associated with colorectal cáncer

Jossimar Coronel Hernández, Eduardo López Urrutia, Verónica García Castillo, Samuel Trujano Camacho y Carlos Pérez-Plasencia.

Unidad de Biomedicina. Facultad de Estudios Superiores Iztacala. Universidad Nacional Autónoma de México.

Avenida de los Barrios Número 1, Colonia Los Reyes Iztacala, Tlalnepantla, Estado de México, C.P 54090. carlos.pplas@gmail.com

Colorectal cancer (CRC) is the fourth most common cause of death cancer-related worldwide. Many studies have shown decreases in the expression of tumor suppressor genes such as p53, GSK3B, APC, CDKN1A, PTEN and Rb1. Moreover, new evidence suggests that microRNAs have an oncogenic role and they could promote cancer trough regulation of tumor growth, proliferation, angionesis and metastasis. Through bioinformatic analysis, we identified mir-26a as a potential regulator of GSK3B, APC, PTEN and Rb1. To analyze the relation between mir-26a and its potential target genes, we measured them in several CRC cell lines and the non-tumoral cell line CRL1790, and we found mir-26a overexpression in CRC cell lines and an inverse correlation with GSK3B, APC, PTEN and Rb1 mRNA and protein expression. Luciferase expression assays demonstrated miR-26a regulated these genes, more significatively PTEN and GSK3B. Finally, we found that overexpression of mir-26a increased proliferation and migration in CCR cells. In an inflammation-associated mouse CRC model, we found overexpression of mir-26a corresponding with decreased GSK3B, PTEN and Rb1. Besides, we observed the same results in paraffin-embedded tissues from Mexican CRC patients. Searching upstream in the regulation pathway, we found that HIF-1a stability seemed to increase mir-26a expression and PTEN downregulation. This fact suggest that HIF-1a is a specific transcription factor for mir-26a, thus rendering it as an "*hypoxi-mir*". Here, we highlight the importance of mir-26a as a regulator in CCR development and suggest it as a target for CCR prevention or therapy.

Area: Genética, Epigenética y Regulación Genética
Jossimar Coronel Hernández: jossi_thunders@hotmail.com Tel: 5522649309

Evaluation of the expression level of miR-132, -203 and -212 in breast cancer tissue and its possible role as post-transcriptional regulators of BRCA-1, BRCA-2 and ATM.

Biol. Pablo Cortés Pérez, DR. Carlos G. Pérez Plasencia, Instituto Nacional de Cancerología, Av. San Fernando No. 22, Col Sección XVI, México, CDMX. C.P. 14080 Tel. 56280400, Email. Carlos.pplas@gmail.com

Breast cancer is a molecular disease characterized by the formation of tumor masses in the tissue of the mammary gland, as a result of uncontrolled cell proliferation. Of 100% of breast cancer patients, 17% is classified as triple negative subtype, which is the most aggressive subtype, patients with this subtype have a high rate of metastasis, being the most common target organs brain and Lung, combined with this characteristic, have the worst prognosis because their overall survival rate is less than 5 years (Sandhu et al., 2010).

Several studies have shown that there is an interaction between the signaling pathway of BRCA-1, BRCA-2 and ATM and breast cancer; (Antoniou et al., 2003); The proteins encoded by these genes play a fundamental role in the mechanisms of response to DNA damage (DDR), since the overexpression or under-expression of these genes promotes progression and genomic instability inheriting an aberrant cell phenotype, causing the tumor cells be resistant to DNA damage caused by various therapies and treatments. (Cordero et al., 2015).

The regulation of gene expression can be regulated by epigenetic mechanisms; such is the case of microRNAs, small non-coding RNA molecules of 22 to 25 nucleotides that play a crucial role as post-transcriptional regulators, when they are bound by complementarity to the 3'UTR region of the mRNA.

When performing a bioinformatic analysis, 3 microRNAs miR-132, -203 and -212 were obtained that can regulate the mRNA of interest of BRCA-1, BRCA-2 and ATM.

An experimental model was proposed in vitro using triple negative breast cancer cells BT-20 and MDA-MB-231, which were treated with two doses of UVc, for BT-20 a dose of 58 J was used and for MDA- MB-231 of 78 J. In order to corroborate DNA damage, the comet assay was performed using the UVc doses corresponding to each cell line.

To establish the possible interaction between the miRNAs and the mRNA, a real time of the miRNAs -132, -203 and -212 was observed observing a relationship that allows us to infer that the miRNAs can be regulators of BRCA-1, BRCA-2 and ATM.

The overexpression of the BRCA-1, BRCA-2 and ATM mRNAs causes the cells to be resistant to DNA damage with MDA-MB-231 being more resistant in comparison with BT-20.

CHARACTERIZATION OF EPIGENETIC CHANGES ASSOCIATED WITH THE METABOLIC MEMORY *IN VITRO.*

Martí David Wilson Verdugo & Julián Valdés Rodríguez.
Departamento de Biología Celular y del Desarrollo
Instituto de Fisiología Celular, U.N.A.M.
Circuito Exterior s/n Ciudad Universitaria, Coyoacán, 04510. Ciudad de México.
Tel. +52 55 5622 57 27. E-mail: julian.valdes@ifc.unam.mx

Patients diagnosed with diabetes mellitus can achieve good control of blood glucose levels with the aid of pharmacologic intervention and lifestyle changes. However, even with good and prolonged glycemic control, the incidence of chronic diabetes complications in these patients is high in comparison to healthy individuals. This phenomenon is termed ~~"m~~metabolic memory" to point out that short periods of hyperglycemia (months to years) can produce long-term consequences. Current evidence indicates that transient hyperglycemia can cause persistent alterations in gene expression and oxidative stress levels, which in turn can have an impact years, or even decades after glucose levels are normalized.

The fact that short periods of high glucose can induce persistent changes in gene expression, directly points to the possibility that transient hyperglycemia could generate long-lasting epigenetic changes in the chromatin structure, which may have an active role in the pathology of metabolic memory. Indeed, there are some evidences indicating that changes in the epigenome participate in the metabolic memory, but its contribution has not been explored in depth. For the above-mentioned, this study has the goal of characterize the changes in chromatin structure in hyperglycemia and metabolic memory *in vitro*, using human umbilical vein endothelial cells (HUVEC) as model of study.

Our results so far indicate that, exposure of HUVEC to high glucose causes differential expression of some genes, particularly of inflammation mediators, and these changes persist after normalization of glucose levels. Similarly, the same treatment in HUVEC causes a significant increase in oxidative stress levels, which persist after normalization of glucose levels. These results indicate that we have reproduced the metabolic memory phenomenon *in vitro* in HUVEC. Now we are characterizing the changes in chromatin accessibility at genome-wide scale induced by hyperglycemia and metabolic memory, this by using the assay for transposase accessible chromatin coupled with high throughput sequencing (ATAC-seq), as well as an RNA-seq to characterize the differentially expressed genes in the previously mentioned conditions.

Second-site suppressors of mutations in essential genes RHE_PE00001 and RHE_PE00024 of *Rhizobium etli* CFN42 support a role in peptidoglycan synthesis

Araceli Dávalos[1], Sofía Martínez-Absalón[1], Carmen Guadarrama[1], Victor González[2], Patricia Bustos[2], Susana Brom[1] and David Romero[1]

Programas de Ingeniería Genómica[1]y Genómica Evolutiva[2]; Centro de Ciencias Genómicas UNAM Ave. Universidad 2001, Col. Chamilpa. 62210 Cuernavaca, Mor., México. Tel. (777) 3175867; davalos@ccg.unam.mx

Rhizobium etli CFN42 is a nitrogen-fixing bean symbiont with a genome composed of a chromosome and six large plasmids (p42a-p42f). A systematic deletion analysis revealed two novel essential genes (RHE_PE00001 and RHE_PE00024) on p42e, validating its designation as secondary chromosome. Conditional knockdown (cKD) mutations in both genes revealed an interesting phenotype, characterized by a high proportion of round cells, instead of the normal bacillary form. Both cKD mutants display slower duplication times than the wild type and, additionally, the cKD mutant in RHE_PE00024 displays cells that are 30% smaller than wild-type cells. These results indicate that both genes participate in the control of cell division and shape.

Interestingly, it was possible to obtain knockout mutations in both genes, albeit at very low frequencies (10^{-8}-10^{-9}). Under the hypothesis that these knockout mutants contain suppressor mutations, we subjected them to whole genome sequence. Genome analyses of these mutants revealed SNPs that affect two genes in all six mutants. One affected gene is RHE_CH03804, the third gene of an operon preceded by *ftsE* and *ftsX*.

Five of the mutants displayed a V101F change in the predicted protein sequence of RHE_CH03804, while the remaining mutant has a C124Stop change. *ftsE* and *ftsX* participate in peptidoglycan synthesis at the division region. Interestingly, knockout mutations in RHE_CH03804 were very difficult to obtain (10^{-9}), indicating its possible essentiality. A cumate-dependent, cKD mutant in RHE_CH03804 shows in the absence of cumate a significant proportion (9%) of round cells and a novel phenotype, characterized by near-bacillary cells with a bulge in the middle (16%). Both phenotypes were augmented in the presence of cumate (22% round cells, 52% bulging cells), conceivably by increasing RHE_CH03804 gene expression above wild-type levels.

The other region affected in suppressor mutants is in the intergenic region of divergent genes *rne* (encoding ribonuclease EG) and *amiC* (encoding an amidase that cleaves septal peptidoglycan, allowing daughter cell separation). The change is an A-C SNP, that probably affects the expression of one or both of these genes. To evaluate this, suppressor mutants were evaluated for *rne* and *amiC* expression by qPCR. No significant changes were observed for *rne* gene expression, but the suppressor mutants displayed a marked reduction (to just 13-18% of wild-type levels) in *amiC* expression.

These data are consistent with a view in which RHE_PE00001 and RHE_PE00024 affect activities relevant for cell division, that are compensated by modifications in peptidoglycan synthesis and processing.

Relationship between chromatin structure and gene expression linked to stress response in *Debaryomyces hansenii*

Ileana de la Fuente Colmenares, Viviana Escobar Sánchez, Claudia Segal Kischinevzky.
Laboratorio de Biología Molecular y Genómica, Facultad de Ciencias, UNAM Circuito exterior s/n, Ciudad Universitaria CP 04510, Ciudad de México, Tel: 56 22 48 31, e-mail: ile.delafc@gmail.com

All aerobic cells are subjected in some extent to oxidative stress. To overcome the toxicity of oxygen reactive species (ROS), different cells deploy a very conserved response, known as general stress response (GSR) in which it is known that genes that code for antioxidant enzymes such as catalase or superoxide dismutase (SOD) are up-regulated at the transcriptional level in order to achieve physiological adaptation and hence survival to a stressful challenge. The GRS is also activated under osmotic stress conditions because osmotic stress leads to an increase in ROS which may cause oxidative stress.
Debaryomyces hansenii is a non-conventional yeast that has the ability to grow in media containing up to 4 M of NaCl, it also has a great resistance to ROS like H_2O_2 or O_2^-, and it has been previously reported that this inherent resistance to osmotic and oxidative stress stimuli are correlated with a high basal catalase activity compared to *S. cerevisiae* in the same growth conditions, among a milieu of other enzymes and proteins that seem to be adapted to salty environments.

When NaCl is present in high concentrations, it produces an osmotic imbalance between extracellular and intracellular microenvironments. In yeast, ethanol is an important source of osmotic stress due to its less polar character than water, it intercalates in the membrane and induces electron leakage that leads to ROS formation. On the other hand when H_2O_2 concentration rises, it produces oxidative damage to proteins, lipids and in presence of metal ions as Fe, can lead to the most toxic ROS OH^- by Fenton reactions. Because of this, we choose these three stimuli to characterize *D. hansenii* antioxidant enzymes induction by measuring rates of transcription of catalase and SOD genes after a shock at sublethal concentrations of them.
Once we find the sublethal doses of NaCl, H_2O_2 and ethanol by viability assays after an osmotic or oxidative shock, we will quantify the expression of catalase genes (*Dh*CTA and *Dh*CTT) and superoxide dismutase (*Dh*SOD) genes of *D. hansenii* with RT-PCR to find out contrastant conditions of expression and their role in physiological adaptation for this yeast. If RT-PCR results indicates that there is a strong activation or repression of the genes tested under the selected conditions, we will look if there are important changes in nucleosome occupancy at the promoters of these genes using the nucleosome scanning assay (NuSA), this may help us to identify which regions of the promoter are relevant to the activation of transcription of antioxidant enzyme genes in *D. hansenii*.

Estradiol effects on EZH2 expression in human glioblastoma cells

Aylin Del Moral-Morales[1], Ana Gabriela Piña-Medina[2], Ignacio Camacho-Arroyo[1]
[1]Unidad de investigación en Reproducción Humana, Instituto Nacional de Perinatología-Facultad de Química, UNAM. 2Facultad de Química, Departamento de Biología, UNAM.
[1]camachoarroyo@gmail.com Facultad de Química, UNAM, Ciudad Universitaria, Coyoacán, Ciudad de México, C.P. 04510 Tel 56223732

ABSTRACT

Enhancer of zeste homolog 2 (EZH2) is a methyltransferase subunit of the Polycomb repressive complex 2 (PRC2) which catalyzes the mono- di- or trimethylation of histone 3 at lysine 27 (H3K27me3). This epigenetic mark has been associated with the transcriptional repression of tissue-specific genes. It has been reported that EZH2 is overexpressed in several types of cancer including glioblastomas, the most frequent and aggressive primary brain tumors. EZH2 overexpression contributes to glioblastoma progression. It has been shown that sex hormones such as estradiol (E2) increases proliferation of glioblastoma cells and promotes expression of genes through estrogen responsive elements located in promoter sequences. These elements have been reported in the promoter region of EZH2 gene suggesting that its expression should be regulated by E2. To test if E2 could regulate EZH2 transcription, we first determined its basal expression in three cell lines derived from human glioblastomas (U251, U87, and D54). We found that all cell lines differentially express EZH2. U251 cells expressed the highest levels, whereas U87 the lowest. We then treated U251, U87 and D54 cells with E2 10 nM for 3, 6, 12 and 24 hours and evaluated the expression of EZH2 by RT-qPCR and Western Blot. Our data show that E2 increases EZH2 mRNA expression in U87 and D54 cells after 12 hours of treatment, but no significant changes were observed on U251 cells. These data suggest that EZH2 is regulated by E2, and that this effect depends on the cell line context.

Rhizobium genomic edition using the CRISPR / Cas9 system.

Rafael Díaz Méndez[1], Gonzalo Torres Tejerizo[2], David Romero[1].

[1]Programa de Ingeniería Genómica, Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México, Av. Universidad 1001, Col. Chamilpa, 62210 Cuernavaca, Morelos, México. Tel. (777) 3175867; rdiaz@ccg.unam.mx
[2]IBBM (Instituto de Biotecnología y Biología Molecular), CCT-CONICET-La Plata, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Calles 47 y 115 (1900) La Plata, Argentina.

Rhizobium is a genus of Gram-negative soil bacteria with the ability to fix atmospheric nitrogen. *Rhizobium* establishes a symbiotic relationship with leguminous plants. This relationship leads to the establishment of specialized structures called nodules. In these structures the bacteria are able to convert atmospheric nitrogen into ammonia Due to these essential biological characteristics of *Rhizobium*, it is very important to implement novel genetic methodologies that allow us to systematically modify this interesting group of bacteria.

The versatility and easy application of the CRISPR /Cas9 system constitute an interesting alternative for best systematic genetic modification of *Rhizobium*. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated Cas proteins are essential in adaptive immunity in some bacteria and archaea, enabling the organisms to eliminate the attack of invading genetic material. CRISPR/ Cas9 system from *Streptococcus pyogenes* generates permanent mutations by introducing double stranded breaks on invading DNA, these breaks activate the endogenous repair pathways of the cell, resulting in a previously designed specific genetic modification (1).

The resulting double-strand breaks are commonly repaired by homologous recombination, using either identical or altered templates for recombination.

Alternatively, some bacteria (including *Rhizobium*) possess a mutation-prone Non Homologous End Joining (NHEJ) system, allowing the introduction of mutations at the site of the break.

Initial efforts with this system were hindered by the lethality of some Cas9 variants even in the absence of a leader RNA. For instance, cloning of a plant-optimized Cas9 under the control of a *lacZ* promoter resulted lethal in Escherichia coli, even in the absence of a leader RNA. To circumvent this problem, we are using a Xanthomonasoptimized Cas9. A binary system of complatible plasmids will be used. One plasmid harbors the leader RNA and the donor sequence, and the other contains Cas9 under the control of an inducible promoter.

Preliminary results will be presented corresponding to the construction of the CRISPR / Cas9 binary system, as well as the *Rhizobium* behavior containing Cas9 and the first steps of CRISPR / Cas9 *Rhizobium* genetic edition.

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Negative Regulation of Serine Threonine Kinase 11 (STK11) through miR-100 inhead and neck cancer

Gabriela Figueroa González, José Federico Carrillo Hernández, Jossimar Coronel Hernández, Alma Delia Campos Parra, Miguel Rodríguez Morales, Octavio Daniel Reyes Hernández, Eduardo López Urrutia, Nadia J. Jacobo Herrera, Carlos Pérez Plasencia.

Laboratorio de Genómica, Instituto Nacional de Cancerología, México, San Fernando 22, Tlalpan, S. XVI, 14080 México City, México.
Tel. 55 39 00 58 99 ; e-mail: gabufg@gmail.com

Serine Threonine Kinase 11 (*STK11*), also known as Liver Kinase 1 (LKB1), is a tumor suppressor gene which regulates several biological processes such as apoptosis, energetic metabolism, proliferation, invasion, and migration. *STK11* expression may be regulated through epigenetic, transcriptional and post-translational mechanisms. In the present study we analyzed 60 cases of head and neck cancer (HNC) tumor samples and 10 healthy tissues counterparts. *STK11* expression levels and its promoter methylation status was determined in tumor biopsies. Our findings showed that *STK11* was under-expressed and its promoter region was demethylated or partially methylated.

Afterwards, we sought to identify candidate miRNAs exerting post-transcriptional regulation of *STK11* . miR-17, miR-106a, and miR-100 were proposed as negative regulators of *STK11* ; however, our data provided evidence that miR-100 was over-expressed in tumor samples. Finally, we demonstrated by means of luciferase gene reporter assay that miR-100 targets at least two regions located at the 3'-UTR of *STK11* mRNA directly. This is, to our knowledge, the first report of miR-100 targeting *STK11* in HNC. Together, these findings may support the importance of regulation of *STK11* through miR-100 in HNC and the possible contribution to carcinogenesis process in this neoplasia.

Role of Transcription Factor NusG in the Adaptive Mutagenesis of *Bacillus subtilis*

Guillermo de Jesús Flores Álvarez, Hilda Cecilia Leyva Sánchez, Mario Pedraza Reyes.
Departamento de Biología, Universidad de Guanajuato, Noria Alta S/N, Guanajuato, Guanajuato, 36050, MÉXICO. | pedrama@ugto.mx | Tel. (473) 732 0006 (Ext. 8161)

To assess survival and perpetuate life, all organisms must preserve the integrity of the genetic information of their DNA. This essential biomolecule is highly reactive and therefore, susceptible to modifications by endogenous and exogenous agents. However, cells are equipped with complex and sophisticated mechanisms of DNA repair, checkpoint proteins and cell death pathways, which collectively work to reduce the harmful consequences of DNA damage. It has been shown that ionizing and ultraviolet (UV) radiations, starvation and oxidative stress, influence a bacterial process termed Stress-Adaptive or Stationary-Phase-Associated Mutagenesis (SPM)[1]. This phenomenon, which occurs in a population of non-dividing bacteria, subjected to non-lethal selective pressure, elicites genetic variability with a direct impact in survival and evolution[2]. The contribution of transcriptional processess in modulating mutagenic processes has been demonstrated in microorganisms that actively replicate their genetic material, as well as in cells without division. *Bacillus subtilis* possesses 7 distinct transcriptional factors including NusG. This protein plays a fundamental role during the elongation process of the RNA polymerase, reducing the level of pause, and, therefore, increasing the overall rate of transcription[3 4]. A gain-of-function mutagenesis system that employs the strain *B. subtilis* YB955 to determine the production of revertants to the chromosomal auxotrophies *hisC952*, *metB5* and *leuC427* was efficiently employed to study SPM in *B. subtilis*[2].

Employing this SPM system our results revealed that, in reference to the parental strain YB955, a null *nusG* mutant exhibited a significant decrease in the production His^+, Met^+ and Leu^+ revertants. As these observations took place in starving conditions, we concluded that NusG promotes mutagenic events putatively associated with transcriptional events in stationary phase non-replicating bacteria. In agreement with this notion, whereas a transcriptional *nusG-lacZ* fusion was expresssed during all the stages of the growth cycle of *B. subtilis*; the major transcription took place during the stationary phase of growth.

These results support the hypothesis that chromosomal alleles that are highly transcribed in starving *B. subtilis* cells, employs NusG to propitiate mutagenic events which allows them to generate genetic variability and escape from growth limiting conditions.

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Evaluation of MMP2, Notch-1 and Snail1 in siHa cells transfected with members of the miR-34 family

Ixamail Fraire Soto[1], Jesús Adrián López

[1]Laboratorio de microRNAs, Unidad Académica de Ciencias Biológicas, Universidad Autónoma de Zacatecas.
Av. Preparatoria S/N, Zacatecas 98066, México. jalopez@uaz.edu.mx.

Cancer development is regulated by coding and non-coding genes like microRNAs (miRNAs). The aberrant expression of miRNAs is recognized as a molecular mechanism that leads to carcinogenesis. In cervical cancer (CC) the E6 oncoprotein expression promotes p53 degradation, therefore the decrease of effector genes like miR-34 family formed by a, b and c. Each pre-miRNA generates the arms 5p and 3p. The arms 5p and 3p of miR-34a, b and c potentially regulate different genes. The targets of the miR-34 family include Notch-1, Snail1 and MMP2. Notch-1 expression increases proliferation, migration, invasion and inhibits apoptosis. Snail1 is involved in the differentiation of stem cells permitting generation or inhibition of dedifferentiated phenotypes. MMP2 is a zinc-dependent endodipase capable of degrading extracellular matrix and participates in several cellular processes such as proliferation, migration, differentiation and angiogenesis. In this work we evaluated the levels of MMP2, Notch-1 and Snail1 on SiHa cell line transfected with members of the miR-34 family. MiR-34 family members over-expression diminish Notch-1, Snail1 and MMP2 expression, suggesting that these miRNAs could regulate tumorigenic progression in CC.

High glucose-diet reduces *C. elegans* longevity by autophagy in an HLH-30/TFEB dependent manner

Berenice Franco-Juárez, Fanny Mejía-Martínez, Elizabeth Moreno-Arriola, Alain Hernández-Vázquez, Saul Gómez-Manzo, Jaime Marcial-Quino, Antonio Velazquez-Arellano and Daniel Ortega-Cuellar *
Unidad de Genética de la Nutrición, ***Instituto de Investigaciones Biomédicas UNAM*** - Instituto Nacional de Pediatría, Mexico City 04530. dortegadan@gmail.com
*** Author to whom correspondence should be addressed.**

ABSTRACT

High-glucose diet (HGD) is associated with the development of metabolic diseases, including obesity and type-2 diabetes (T2D), that shortened the life expectancy, however the mechanism through it does so is not well understood. Autophagy, an evolutionarily conserved mechanism, has been shown that promotes both cell and organismal survival. The goal of this study was to determine whether exposure of *C. elegans* to HGD affects autophagy and thus contributes to the observed lifespan reduction in HGD. Unexpectedly, nematodes exposed to HGD, results an increased autophagic flux, via an HLH-30/TFEB-dependent mechanism, since that animals with loss of HLH-30/TFEB, even in high glucose exposition, lead to extend of lifespan, suggesting that HLH-30/TFEB might promote detrimental effects on longevity through autophagy under this stress condition. Interestingly, pharmacological treatment with okadaic acid, an inhibitor of PP2A or PP1 protein phosphatases, blocks HLH-30 nuclear translocation, but not the TAX-6/calcineurin suppression by RNAi, during glucose exposure. Together, our data support the suggested dual role of HLH-30/TFEB and autophagy, that depending cellular context, may promote either organismal survival or death.

Stress induced expression of *SLM35* in *Saccharomyces cerevisiae*

Hernán Romo-Casanueva & Soledad Funes
Departamento de Genética Molecular, Instituto de Fisiología Celular, UNAM
Circuito Exterior s/n, Ciudad Universitaria, Coyoacán Cd.Mx. 04510
(+52-55) 56225679, sfunes@ifc.unam.mx

The yeast mitochondrial protein Slm35 (*Stress and Longevity-related Mitochondrial Factor*) is functionally related to the TOR signaling pathway. A mutant lacking *SLM35* shows a resistance phenotype to oxidative and thermal stress, similar to what has been observed before for a Δ*tor1* strain. In contrast, the simultaneous deletion of both genes results in a strong sensitivity to these stresses as well as a strong decrease in lifespan. Furthermore, *SLM35* genetically interacts with components involved in autophagy and negatively regulates the mitophagy flux. In addition, the protein Slm35 is predicted to posses a scramblase domain, which would imply that this protein can modulate the distribution of phospholipids in biological membranes in response to stress, however, experimental evidence to support this hypothesis is not yet available.

Little is known of what exactly the protein does or how it acts with Tor1; furthermore, it is not known whether Slm35 is expressed constitutively or in response to an external stimulus.This work seeks to determine the transcriptional regulation of the *SLM35* gene in response to oxidative stress in *S. cerevisiae*. In order to understand this, we used a transcriptional reporter in which the expression of the *LacZ* gene of *E. coli* is controlled by 1000 bp of the 5'-UTR of *SLM35.* We transformed mutant strains of several regulatory proteins involved with the stress response in yeast with this plasmid, and monitored the activation of the promoter by measuring the activity of β-galactosidase in 0.75 mM of *ter*-butyl hydrogen peroxide.

Through our studies, we have concluded that the *SLM35* promoter responds to oxidative stress by diminishing its rate of expression in a time-dependent fashion. Nevertheless, we observed that by eliminating *TOR1* the activity of the enzyme is larger than the activity in the wild type strain. This indicates a possible regulation by the transcriptional factors downstream to Tor1, like Msn2, Msn4 and Gis1, in which Tor1 impedes their translocation to the nucleus. When we analyzed the activity of the reporter in the *Δmsn2* and *Δmsn4* strains, we observed a reduction of activity in oxidative stress stimulus as compared with the wild type strain in the same conditions. With the double mutant *Δmsn2Δmsn4* we see a reduction of activity in oxidative stress as well, but the activity in normal conditions is larger than the single mutants. Interestingly, the mutant *Δgis1* is not affected by oxidative stress and has a larger activity than any other strain indicating that Gis1 is the transcription factor that regulates our promotor.

Even though we see an effect in the promoter's activity when the Tor1 pathway is affected there is still a considerable reduction of activity in oxidative stress conditions, indicating there is another signal that is repressing the *SLM35* promoter. At the same time, it is surprising that Gis1 is inhibiting our promoter seemingly independent of Msn2/4. Suggesting that *SLM35* has a more complex regulation than initially thought.

Cysteine-rich receptor-like kinase gene family identification in *Phaseolus* genome and functional characterization of *PvCRK26* and *PvCRK46* during rhizobial symbiosis

Gabriel-Xicoténcatl García [1], Elsa-Herminia Quezada [1], Manoj-Kumar Arthikala[1*], Miguel Lara [2] and Kalpana Nanjareddy [1]
[1] Ciencias Agrogenómicas, Escuela Nacional de Estudios Superiores Unidad León—Universidad Nacional Autónoma de México (UNAM), León C.P. 37684, Mexico.
[2] Departamento de Biología Molecular de Plantas, Instituto de Biotecnología, Universidad Nacional Autónoma de México (UNAM), Cuernavaca C.P. 62271, Morelos, Mexico.
*** Correspondence:** manoj@enes.unam.mx ; Tel.: +52-477-194-0800 Ext.43462

RLKs are conserved upstream signaling molecules that regulate several biological processes, including plant development and stress adaptation. Cysteine (C)-rich receptor-like kinases (CRKs) are an important class of RLKs, which play vital roles in disease resistance and cell death in plants. Genome-wide analysis of *CRK* genes have been carried out in *Arabidopsis* and rice while, functional characterization of some CRKs have been realized in wheat and tomato besides *Arabidopsis*. A comprehensive analysis of the *CRK* gene family in leguminous crops has not yet been conducted, and our understanding of their roles in symbiosis is rather limited. Here, we report the comprehensive analysis of the *Phaseolus CRK* gene family including identification, sequence similarity, phylogeny, chromosomal localization, gene structures, transcript expression profiles and *in silico* promoter analysis. Forty-six CRK homologs were identified which were phylogenetically clustered into five groups. Expression analysis suggests that *PvCRK* genes differentially express in both vegetative and reproductive tissues. Further, transcriptomic analysis revealed several *CRK* genes being upregulated during rhizobial symbiosis. Among them *CRK26* and *CRK46* are highly upregulated during nodule formation. Functional characterization of these CRKs by RNAi technology in hairy roots of *Phaseolus* show symbiosis phenotype i.e., infection thread abortions and poor nodule numbers. Together, we conclude that *CRK26* and *CRK46* are essential for nodule formation in *Phaseolus*.

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Sodium butyrate effect on growth and dimorphic transition of *Yarrowia lipolytica*

Juan Manuel González Prieto[1], Christian Alfonso Arenas[2], María Cristina Herrera[2], Sandra Milena Leal Pinto[2] and Viviana Roa Cordero[2]
[1]Biotecnología Vegetal. Centro de Biotecnología Genómica. Instituto Politécnico Nacional. Blvd. Del Maestro s/n. esq. Elías Piña. Col. Narciso Mendoza. C.P. 88710. Reynosa, Tam. México. +8999243627. jmgpcbgipn@gmail.com
[2]Universidad de Santander UDES. Calle 70 N° 55-210, Bucaramanga, Santander, Colombia. +5776516500 Ext 1214

Epigenetic modifications on nucleosomal histone tails are involved in regulation of gene expression. Acetylation is one of the most important epigenetic marks which correlate with gene activation, and this is a reversible modification mediated by histone acetyl transferases (HAT) and histone deacetylases (HDAC). Inhibitors of HDAC (HDACi) induce hyper-acetylation and an increase in gene activation. Sodium butyrate (NaBut) is a well known short chain fatty acid which inhibit HDACs.

In order to study the role of histone acetylation on fungal dimorphism, we assayed the effect of NaBut on *Yarrowia lipolytica* P01a strain. The kinetic growth of P01a strain in Yeast Nitrogen Base (YNB)-glucose medium with different concentrations of NaBut (0, 1.25, 2.5, 5.0, 10, 20, 40, 60, 80, 100, 250 and 500mM) were tested. The cultures were grown in flask during 40 hours with rotary agitation at 250rpm. Cell densities were measured by cell counts, stained with Trypan Blue at 8-hour intervals. To test the effect of inhibitor on yeast to micelial transition we used YNB medium supplemented with N-acetyl-D-Glucosamine (1%) instead glucose and buffered with 100mM citric acid adjusted to pH 7.0, both as effectors of morphogenetic switch. At least 200 cells were counted, discriminating between filaments and yeasts, after 16 hours of growth. The results were expressed as percentage of mycelium. We found that 250mM of NaBut significantly supressed *Yarrowia lipolytica* filamentation (0,1%; $p<0,01$). By contrast, 53% of the cells gave filaments in absence of inhibitor. It is noticeable that at lower concentrations than 250mM, growth promoting effect in a dosis dependent manner, was observed.

Our results demostrated that *Yarrowia lipolytica* is able to utilize NaBut as sole carbon source due to its lipolytic activity. However once metabolic capability is overcome, NaBut produces arrest of dimorphic transition, which suggest that acetylation of histones plays a pivotal role in regulating this process.

Analysis of SNP's in the *Pun1* Gene (*Capsaicin synthase*) in some cultivars of *Capsicum annuum* of Mexico

Alberto González Zamora[1*], Clarita Olvera Carranza[2] and Rebeca Pérez Morales[3].
[1] Laboratorio de Biología Evolutiva, Facultad de Ciencias Biológicas, Universidad Juárez del Estado de Durango, Gómez Palacio, Durango, México
[2] Departamento de Ingeniería Celular y Biocatálisis, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, México
[3] Laboratorio de Biología Celular y Molecular, Facultad de Ciencias Químicas, Universidad Juárez del Estado de Durango, Gómez Palacio, Durango, México
[*] **Autor responsable:** Alberto González-Zamora. Laboratorio de Biología Evolutiva, Facultad de Ciencias Biológicas, Universidad Juárez del Estado de Durango, Gómez Palacio, Durango, México. Tel (871) 7 15 88 10, e-mail: agzfc@ujed.mx.

Abstract: Capsaicinoids are exclusive of the genus *Capsicum* and are responsible for fruit pungency. The genetic basis of pungency is not yet fully understood, although several candidate genes have been analyzed including the *Pun1* gene that encodes an enzyme involved in the metabolic pathway for the synthesis of capsaicinoids. The objective of this study was to analyze the variability of polymorphisms in the *Pun1* gene, in some samples of *Capsicum annuum* of Mexico, adapted to conditions of high temperature. First, RFLP studies were performed with 7 restriction endonucleases and 3 of them: *Alu I, Bsr I,* and *Hinf I,* generated highly variable patterns. When sequenced representative cultivars were examined, we
found 26 single nucleotide polymorphisms (SNPs): 19 in the region corresponding to the first exon and 7 in the intron. Of the discovered SNPs, 6 in the coding region generated amino acid changes. The cultivar Chiltepin showed more than one sequencing product, suggesting that it has more than one copy of the *Pun1* gene. Cultivars of Mexican *C. annuum* analyzed have a high variety of polymorphisms in the *Pun1* gene that regulate the biosynthetic pathway of major capsaicinoids and this variability is found at the DNA and protein levels.

Functional diversification of paralogous α-*Isopropylmalate synthases* from the ancestral type yeast *Lachancea kluyveri.*

Estefany Granados Avalos, Guadalupe Vigueras Menenses, Carlos Campero Basaldúa, Beatriz Aguirre López, Alicia González Manjarrez.
Instituto de Fisiología Celular, Universidad Nacional Autónoma de México.Circuito Exterior s/n Ciudad Universitaria, Coyoacán, 04510 Ciudad de México. 525556225631. amanjarr@ifc.unam.mx

Saccharomyces cerevisiae originated from the hybridization of two different yeast species, followed by a whole genome duplication event. It has been hypothesized that the retention and specialization of some paralogous pairs could have influenced or determined the acquisition of facultative metabolism.

S.cerevisiae retained Leu4/Leu9, a paralogous pair of homodimeric α-isopropylmalate synthases (α-IPMS), which catalyze the first step of leucine biosynthesis. Their diversification resulted in different leucine sensitivity to feedback inhibition: Leu4-Leu4 is leucine sensitive, while Leu9 homodimers are leucine resistant. In a *leu4*Δ mutant, Leu9-Leu9 resistant homodimers result in an increased leucine biosynthesis and leucine accumulation, particularly under respiratory conditions. The increase in leucine biosynthesis results in depletion of the Krebs cycle intermediates and poor growth.

This work focuses on the study of functional divergence of the α-IPMS paralogous present in the ancestral type yeast *Lachancea kluyveri*: *Lk*Leu4 and *Lk*Leu4bis, which are Leu4-Leu9 orthologous, which resulted from a sporadic duplication. Deletion of *LkLEU4* (*Lkleu4Δ LkLEU4BIS*) results in poor growth, which resembles a *leu4Δ LEU9* phenotype, while an *LkLEU4 Lkleu4bis*□ mutant displays wild type phenotype. Kinetic characterization of the ancestral type isozymes did not show a significant difference in their kinetic parameters or their leucine sensitivity to inhibition; thus, these enzymes did not diverge in their leucine sensitivity.

Preliminary results show that leucine supplementation of *Lkleu4Δ* and *Lkleu4Δ Lkleu4bisΔ* under respiratory conditions does not restore the wild type growth phenotype. Additionally, *Lkleu4Δ* intracellular leucine and α-IPMS activity quantification *showed wild type values.* Thus, we hypothesize that the poor growth of *Lkleu4Δ* is not the result of low intracellular leucine, but due to a lack of α-isopropylmalate (α-IPM), a leucine precursor, which additionally, functions as a coactivator for the transcription factor Leu3, in its absence Leu3 acts as a repressor. Leu3 is responsible for the transcriptional activation of several of the branched-chain amino acid biosynthetic enzymes and Gdh1 (glutamate dehydrogenase). Therefore, we hypothesize that *Lkleu4Δ* is incapable of restoring its wild type phenotype under respiratory conditions, even when supplemented with leucine, because it exhibits an α-IPM bradytrophy that affects not only leucine biosynthesis, but also that of valine, isoleucine and glutamate.

Genetic evidence for homodimerization of the proteins encoded by essential genes RHE_PE00001 and RHE_PE00024 in *Rhizobium etli* CFN42

Carmen Guadarrama[1], Sofía Martínez-Absalón[1], Araceli Dávalos[1], Lourdes Girard[2], Susana Brom[1] and David Romero[1]
Programas de Ingeniería Genómica[1]y Biología de Sistemas y Biología Sintética[2]; Centro de Ciencias Genómicas UNAM Ave. Universidad 2001, Col. Chamilpa. 62210 Cuernavaca, Mor., México. Tel. (777) 3175867; carmengr@ccg.unam.mx

Rhizobium etli CFN42 is a nitrogen-fixing bean symbiont with a genome composed of a chromosome and six large plasmids (p42a-p42f). A systematic deletion analysis revealed two novel essential genes (RHE_PE00001 and RHE_PE00024) on p42e, validating its designation as secondary chromosome. Conditional knockdown (cKD) mutations in both genes revealed an interesting phenotype, characterized by a high proportion of round cells, instead of the normal bacillary form. These results indicate that both genes participate in the control of cell division and shape. RHE_PE00001 is a hypothetical protein with a DUF1612 domain (domain of unknown function) and a helixturn-helix motif. RHE_PE00024 is a sensor histidine/kinase hybrid protein, participating in a two-component signal pathway. However, the response-regulator protein and the target genes for the signal pathway are as yet unknown.

Dimerization is a frequent characteristic in sensor histidine/kinase and transcriptional regulator proteins (TR). To evaluate the possibility of dimerization of the proteins encoded by RHE_PE00001 and RHE_PE00024, we took advantage of a LexA-based system (1). LexA is a TR whose activity depends on dimerization. The DNA binding domain is located on the NH2 part of LexA, while dimerization depends on the presence of a COOH domain. An *Escherichia coli* cell expressing only the NH2 part of LexA fails to repress the expression of *sulA-lacZ* fusion. Repression can be instrumented by fusing the NH2 part of LexA with an ORF with the ability to dimerize. To advance towards this objective, we generated chimeras containing either the NH2 part of LexA fused with fulllength RHE_PE00001 or the NH2 part of LexA fused with full-length RHE_PE00024.

Both chimeras in *E. coli* displayed a marked repression activity of a *sulA-lacZ* fusion, indicating that both RHE_PE00001 and RHE_PE00024 are able to homodimerize. Interestingly, a few of the clones for both proteins failed to repress the fusion. DNA sequencing revealed that the chimeras able to repress (hence, able to dimerize) contain wild type sequences for either RHE_PE00001 or RHE_PE00024. Chimeras unable to repress harbor DNA changes that affect protein structure (RHE_PE00001, A56V, D135G or S321P and RHE_PE00024, W169R). Experiments are under way to evaluate if these changes affect dimerization ability and/or stability of these proteins. There are modifications of the *lexA* system that allow the evaluation of heterodimerization with possible cognate proteins. Experiments toward this objective will be presented.

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BDNF SNP´s are Associated with Body Composition and Bone Mineral Density in Postmenopausal Women

Guerrero-Contreras Israel[1], Campos-Góngora Eduardo[1], Ramírez-López Erick[1], Velázquez-Cruz Rafael[2], Hernandez-Tobías Esther Alhelí[1], Jiménez-Salas Zacarías[1].
[1]Universidad Autónoma de Nuevo León, Facultad de Salud Pública y Nutrición, Monterrey, México.
[2]Instituto Nacional de Medicina Genómica, Ciudad de México, México.

Introduction: Obesity and being overweight are complex conditions; they are highly hereditary and have reached alarming numbers in Mexico because 70% of the adult population are either obese or overweight. The multifactorial etiology includes: life style, environment and genetics. The Brain derived neurotrophic factor (BDNF) is part of satiety and the energy usage and have a connection with obesity and bone mineral density. (BMD).

Objective: Evaluate the correlation of the polymorphisms; BDNF-rs6265 and rs7934165 of the woman body composition that are postmenopausal that live on the North side of Mexico.

Methods: 182 women were studied, divided into three groups based on the Body Mass Index: normal (N = 36), overweight (N = 75) and obesity (N = 71). Measurements of body composition and BMD were obtained by dual X-ray absorptiometry (DXA). Genotyping was performed with TaqMan probes from DNA extracted from peripheral blood. The statistical analysis included differences in means (ANOVA, Fisher's exact) and the association of variables (linear regression).

Results: In general, the genotype rs6265-TT was associated with a decrease in BMD of the femur (β: -0.05, P ≤ 0.01). The rs7934165-GG genotype showed association with the increase in fat mass index (β: 0.38, P = 0.04).

Conclusion: These results suggest the participation of BDNF in the increase of body fat and the decrease in BMD. Therefore, this gene could be used as a risk biomarker for osteoporosis, obesity and overweight. However, the results should be taken with caution due to the genetic stratification of the population.

CgABF1 is an important regulator of adherence, chronological life span and oxidative estress response in *Candida glabrata*

Ma. Guadalupe Gutiérrez-Escobedo, Grecia Hernández-Hernández, José Oscar Arturo Hérnandez-Carreón, Osney Leyva-Pelaez, José Cruz-Mora, Alejandro De Las Peñas-Nava and Irene Castaño-Navarro*.
Instituto Potosino de Investigación Científica y Tecnológica, AC. Camino a la Presa San José 2055. Lomas 4ª. Sección. 78216. San Luis Potosí, SLP.

Candida glabrata is an opportunistic fungal pathogen that has become increasingly frequent in the last decades in inmunocompromised patients. This yeast adheres to host epitelial cells. The adherence in *C. glabrata* is mediated mainly by Epa1, a cell wall protein, which is part of the Epa family of adhesins. *EPA1* and other adhesin-encoding genes are targets of several levels of regulation: subtelomeric silencing mediated by SIR complex, the protosilencer Sil2126 and the negative element (NE). Abf1 is a transcriptional regulator that has several functions in *Saccharomyces cerevisiae*: Abf1 (ARS binding factor 1) is required for silencing the mating type loci *HML* and *HMR* it is also involved in DNA replication, DNA repair and chromatin organization. We have found that the activity of Sil2126 depends of the C-terminal domain of *CgABF1* to silence a reporter inserted near the telomere E_R and other telomeres. Moreover, *CgABF1* is essential for viability and negatively affects cell growth when overexpressed.

In this work, we characterized the growth rate, chronological life span (CLS), survival to neutrophils and the adherence to HeLa cells of several mutants carrying either the truncation allele *abf1-43*, or an *ABF1* overexpressing plasmid *abf1*Δ/pP_{ABF1}::*ABF1* and *abf1*Δ/pP_{MT1}::Myc-*ABF1*. We found that these mutants grow slower than the parental strain in rich medium, but the biggest delay is observed when *ABF1* is overexpressed in *abf1*Δ/pP_{MT1}::Myc-*ABF1*. Interestingly, we observed that the CLS was increased in all the strains tested (*abf1-43*, *abf1*Δ/p$_{PABF1}$::*ABF1* and *abf1*Δ/p$_{PMT1:}$:Myc-*ABF1*) in comparison with parental strain. When we analyzed the survival to neutrophil attack we found that *abf1-43* and *abf1*Δ/pP_{MT1}::Myc-*ABF1* display the same behavior as the parental strain, while *abf1*Δ/pP_{ABF1}::*ABF1* strain is more susceptible than strain. *abf1-43* is more adherent than wt strain, suggesting that *EPA1* may be derepressed in this strain. These results indicate an important role for *CgABF1* in the control of adherence, CLS and oxidative stress response in *C. glabrata*.

¿Is there a relationship between genetic variability and viability loss of the *Escontria chiotilla* and *Stenocereus pruinosus* (Cactaceae) seeds?

David Alejandro Guzmán-Hernández[1*], Claudia Barbosa-Martínez[1], Juan Manuel Villa-Hernández[1], Joel Marín-Ramírez[1], Francisco José Fernández-Perrino[2], Leticia Ponce de León-García[1].
[1]Departamento de Biología. 2Departamento de Biotecnología. DCBS. Universidad Autónoma Metropolitana-Iztapalapa. San Rafael Atlixco No. 186, Col. Vicentina, Iztapalapa, C.P. 09340, México, CDMX. *dagh0709@gmail.com

Studies of genetic variability in plants have been used to clarify phylogenetic relationships, evaluate the extinction risks of populations and species, propose conservation strategies and compare the effect of domestication. However, there are no studies of genetic variability focused to determinate the seeds viability loss and because of that the longevity is a genetically determined characteristic, molecular analyzes could be useful to explain this physiological process. In this work, the genetic variability of seedlings from *Escontria chiotilla* (F. A. C. Weber) Rose and *Stenocereus pruinosus* (Otto) Buxbaum was compared, considering a possible relationship between this and viability loss of different harvests seeds (2007, 2010 and 2014), both in *ex situ* conditions, as buried *in situ*, with the purpose by contributing to the knowledge of the dynamics of the cactus seed banks. Viability was determined by germination tests in seeds of both species, conserved *ex situ* (25±5 ºC, dark and dry) and, *in situ* in two microsites in a xerophytic scrub in the Valle Tehuacán-Cuicatlán during 24 months. The genetic variability was determined through molecular AFLP markers using 14 combinations of primers, in 21 seedlings of six month old from *E. chiotilla* and *S. pruinosus* coming from seeds of different harvests. The amplified fragments were evaluated by automatic sequencing and the number of polymorphic bands for each combination was quantified. The similarity coefficients were estimated using the Nei and Li index and a dendrogram was constructed for each species. The results showed that the seeds of both species remain viable for more than 8 years in *ex situ* conditions (germination > 89%), while in natural conditions they remain viable for a period of more than one year but less than two, indicating that they have the potential to establish seed banks soil persistent in short term. Otherwise, genetic analyzes showed that 976 bands were amplified in *E. chiotilla*, 132 polymorphic (13.52%) with a similarity index of 0.90 to 0.96, while in *S. pruinosus*, 704 bands were amplified, 72 polymorphic bands (10.22 %) with a similarity index of 0.88 to 0.94. These data suggest that the genetic variability of *E. chiotilla* (endemic and wild species) is 3% higher that of *S. pruinosus* (cultivated species). However, the viability seeds of both species and harvests is similar, both in *ex situ* conditions and *in situ*; Therefore, no relationship was found between viability loss and genetic variability. The viability loss could be due to multiple factors, such as environmental conditions, microorganisms and physicochemical properties of the soil that affect the metabolism of seeds.

Effect of bisphenol A in steroid hormone receptors methylation patterns

Luis Ricardo Hernández Barrientos, Carmen Adriana Mendoza Rodríguez*
Faculty of Chemistry, UNAM
*adrimed@yahoo.com tel. 56223820 Facultad de Química, Edif. F, Lab. 323. Circuito interior s/n Coyoacán C.P.04510 Ciudad de México.

Bisphenol A (BPA) is a high-production-volume industrial chemical that is widely used in the production of polycarbonate (PC) plastics and epoxy resins. These materials are used in many daily consumer products including food containers, water bottles, infant feeding bottles, toys, thermic paper, dental sealants, adhesives and electronic components. Humans are often exposed to BPA through inhalation, dermal exposure and their diet; last one is the principal route of contact because BPA can release from the container to food and beverages. Moreover, the molecular structure of BPA is similar to estradiol, so it is considered an endocrine disruptor compound (EDC). It can bind to steroid hormone receptors like estrogen, progesterone and androgen receptors and change the homeostasis of endocrine system.
In several studies, BPA has been shown to have multigenerational and transgenerational adverse effects on reproductive health even at lower doses than the no observed adverse effect level (NOAEL) (50 mg/kg/day) and the acceptable daily intake (ADI) (0.05 mg/kg/day). These effects are more relevant during critical periods of development of the reproductive tract. Determining which of the different molecular mechanisms mediate the effects of BPA on different aspects of human health is the goal of a considerable amount of research. In this case we study if BPA can alter the expression of sexual hormone receptors and whether the mechanism for achieving this could be changes in the methylation status of the promoter region. For this reason, we focus on studying the effect of perinatal exposure to BPA, in female rats, over gene expression of progesterone and estrogen receptors in their uterus.
Adult female pregnant Wistar rats (F0) were separated in three groups of n = 5 rats/group: BPA low dose (BPA-L) received 0.05 mg/Kg/day, BPA high dose (BPA-H) received 20 mg/Kg/day and the control group only received the vehicle. Experimental females were exposed to BPA in their drinking water from gestational day (GD) 6 to day 21 of lactation. Then, F1 pups were supplied with unadulterated drinking water until 3 months of age and were mated. On GD 6, pregnant F1 dams were administrated with 0.3 mL of 2.0 % Evans blue dye in saline, 20 min before animal sacrifice to detect the implantation sites in the uterus. Implantation sites were cut and divided for DNA, RNA and proteins extractions.
We observed a significant increase of estrogen receptor alpha expression in the BPA-H animals and a significant increase of progesterone receptor expression in the BPA-L animals, as compared to the control. Now we are studying the methylation status of the promoter regions of these genes to evaluate if epigenetic events are responsible of the expression changes.

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AMPK MODULATES THE GENETIC AND METABOLIC EFFECTS OF BIOTIN DEFICIENCY

Alain Hernández-Vázquez; Elizabeth Moreno-Arriola; Marion González-Koll; Daniel Ortega-Cuellar and Antonio Velázquez-Arellano.

Unidad de Genética de la Nutrición, Instituto de Investigaciones Biomédicas, UNAM. Instituto Nacional de Pediatría, Torre de Investigación, 4° piso, Insurgentes Sur 3700, Letra C, Delegación Coyoacán, C.P. 04530. velare@unam.mx. Tel. 56226421

ABSTRACT

Our research group found that the deprivation of biotin essential cofactor interferes with ATP production through the reduction of oxidative phosphorylation (OXPHOS), decreasing mRNAs and proteins necessary for liver lipogenesis and increasing those for fatty acid oxidation. These effects are mediated by the eukaryotic energy sensor AMPK. The changes are accompanied by altered transcription and epigenetic mechanisms, and are reversed by augmenting the cellular energy charge through the supplementation of the corresponding deficient cofactor. The effects are associated with parallel modifications in the corresponding metabolic pathways, e.g., muscular insulin sensitivity modifications mediated directly by AMPK and the glucose transporter GLUT4. We propose that deprivation and supplementation of OXPHOS cofactors are analogous to fasting and feeding, therefore, models to better understand the mechanisms underlying obesity and diabetes after excessive alimentation and life style changes. Our latest research showed that the expression of mRNAs and proteins of FAS and CPT1 are also modulated by the AMPK energy sensor. To evaluate the metabolic flux of fatty acid synthesis, we conducted experiments using [14C] -acetate as precursor and found a significant decrease of the flux under biotin deprived conditions (BtDEF). We also measure the flow in the oxidation of fatty acids by giving [14C] -palmitate and quantifying its incorporation to the CO_2 yield and, contrary to what we expected, we found that the oxidation was lower in biotin deprived conditions. We hypothesize that this is because under BtDEF, the availability of free carnitine is a limiting factor in the incorporation of fatty acids into the mitochondria. We confirmed this by adding carnitine in excess and observed that the flow in the oxidation increased to normal values. The participation of the transcriptional factors SREBP1c and ChERBP was analyzed by means of western blot, we observed that its nuclear localization in the BtDEF is almost null, in addition, a smaller amount was found in the cytosol, which could possibly explain its repression under BtDEF.

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Search of the components of 5' to 3' and 3' to 5' pathways of mRNA decay in *Ustilago maydis*

José Juan Jacinto Vázquez[1], Leticia Vega Alvarado[3], Candelario Vázquez Cruz[2], Estela Anastacio Marcelino[1], Ma. Patricia Sánchez Alonso[2]. [1]Posgrado en Microbiología, Instituto de Ciencias, BUAP. [2] Centro de Investigaciones en Ciencias Microbiológicas, BUAP. Av. San Claudio y 24 sur, edif. 103 J. C.U. 72570. Puebla, Puebla, México. Centro de Ciencias Aplicadas y Desarrollo Tecnológico[3]
Tel. 52 (222) 229 5500 ext. 2523 y 2534 email. maria.sanchez@correo.buap.mx

The control of gene expression is highly regulated in all living organisms. Specifically, the control of transcriptional expression of genes encoding proteins could be through the decay of mRNA. The mRNA decay is a process by which ribonucleic acid molecules are degraded by enzymatic pathways after translation, and comprise two pathways, the 5' to 3' and the 3' to 5' pathways of degradation. Decay of mRNA can occur both in the cytoplasm and in the nucleus, and destined transcripts for degradation involve those which meet their half-life as well as ncRNA, rRNA, and those RNAs that have defects in their sequence or in their structure. The decay of mRNA machinery involves several proteins and elements that act both *in cis* and *in trans*, forming complexes or acting alone, or even in couples for regulation of the degradation process. In *Saccharomyces cerevisiae* some of these proteins involved in RNA decay include complexes such as deadenylation (Pan2-Pan3, Ccr4-Not), and decapitation (Dcp1-Dcp2) complexes, while exoribonucleases (Xrn1, Rrp44) degrade the body of the RNA, Xrn1 degrades the RNA from the extreme 5' to the 3' end, and the exosome complex (composed by nine subunits) degrades the mRNA from the 3' end to the 5' end. In this work we carried out an *in silico* analysis for the search of the putative genes involved in the cytoplasmic RNA decay machinery of the phytopathogen fungus *U. maydis*, but focused on the 5' to 3' and 3' to 5' pathways.
Here we used the transcriptome data of the *U. maydis* laboratory strain 521 (donated by Holloman W.K.) as well as their telomerase-negative mutant strains *trt1-1* (Type 1-like survivor) and *trt1-2* (Type II-like survivor). This fungus lacks the RNAi machinery, besides having a considerable number of genes without introns. Several orthologs of the splicing machinery, as well as others from RNA trafficking and NATs have been documented previously, which makes it an attractive model for the study of RNA metabolism. In these previous analyses it was found that *U. maydis* carried in its postranscriptional machinery some important genes as Ccr4, Dcp1 and Dcp2, Rrp6 as well as Xrn1 essential for the decay of mRNA. However, some genes such as Ski7 and Ski8 were not identified. These genes are required in *S. cerevisiae* for efficient mRNA degradation via the 3' to 5' pathway. Here we report for first time the identification of the putative genes encoding Ski7, Ski8 and Edc3 (an enhancer for decapitation in lane 5' to 3') of *U. maydis* by transcriptome analysis. Those genes are important for both 3' to 5' and for 5' to 3' pathway of mRNA decay. Specific activators are also required, such as the Ski complex (hetero-tetramer Ski2-Ski3-Ski8); in this scenario the Ski7 protein is required to form a bridge between the exosome and the Ski complex. In the yeast cytoplasm the remnants of the transcripts with its cap (m7Gppp) are degraded by the enzyme Dcs1, which has also been identified in this fungus. Previous reported data in other fungi suggest that the components of 3’ to 5’ RNA decay pathway are needed for adaptation to stress and for pathogenicity of *U. maydis.* The expression Ski encoding genes is currently checked by qRT-PCR and discussed our results.

A member of ANR family modulates the expression of genes regulated by PerA in enteropathogenic *Escherichia coli*

Juan Bernardo Jaramillo-Rodríguez[1], María Lilia Cedillo-Ramírez[2], Jorge Alberto Girón Ortiz[2], Ygnacio Martínez-Laguna[3], Cristina Lara-Ochoa[2*]
[1]Licenciatura en Biomedicina-BUAP, 2Centro de Detección Biomolecular-BUAP, 3VIEP-BUAP, *Boulevard Valsequillo s/n. C.P. 72592. Puebla, Puebla. clara_ochoa@hotmail.com

Enteropathogenic *E. coli* (EPEC) is one of the main causes of diarrhea in children in developing countries. EPEC infections are characterized by two distinctive phenotypes: localized adherence (LA) and formation of attaching-and-effacing lesions (A/E). The genetic components required for these phenotypes are encoded mainly within the EAF plasmid (EPEC adherence factor) and the pathogenicity island LEE (locus of enterocyte effacement). The regulation of EPEC virulence genes are under the effect of diverse regulators, one of which is PerA, a protein belonging to the AraC/XylS family. PerA activates its own expression and that of the *bfp* operon involved in the formation of the bundle-forming pilus (BFP) that mediates the LA phenotype; PerA also promotes PerC expression, which in turn stimulates LEE activation through the LEE-encoded regulator Ler.

In 2014, Santiago *et al*., reported the existence of a new family of anti-virulence regulators which they called ANR (AraC-family Negative Regulator) that suppress the effects of AraC/XylS members in Gram-negative. Aar (AggR-activated regulator), a prototype member of this ANR family in enteroaggregative *E. coli* interacts with AggR, a AraC type regulator that activates several virulence genes expression; this interaction prevents AggR from binding to DNA, avoiding its function as an activator.

In this study we evaluated the effect of the *orfRS14140* (a homologue of *aar*) on the expression of virulence genes regulated by PerA in EPEC. For this aim, we examined in EPEC wild type the effect *orfRS14140* overexpression on *perA*, *bfpA*, *perC* and the genes *espB* and *escJ* encoded in LEE by qRT-PCR. Our results suggest that *orfRS14140* represses expression of *perA*, *bfpA*, *perC*, *espB* and *escJ.* At the same time, we analyze overexpression effect of this putative ANR on secreted LEE proteins and on the BfpA and EspA expression in total extracts by western blotting. *orfRS14140* represses the secretion of proteins of LEE and also the expression of BfpA and EspA whereas DnaK, a protein used as a control was not affected.

The results of this work suggest that *orfRS14140* modulates negatively the expression of virulence factors regulated by PerA. The existence of this anti-virulence protein in EPEC indicates the importance of this ANR family in control of virulence attenuation in order to favor the survival of the pathogen.

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Interaction of the BER pathway with Transcriptional Factors Mfd and GreA and its consequences on *Bacillus subtilis* Adaptive Mutagenesis

Hilda Cecilia Leyva-Sánchez, Mario Pedraza-Reyes
Departamento de Biología, Universidad de Guanajuato, Noria Alta, Guanajuato, Guanajuato, CP 36050, México | pedrama@ugto.mx | Tel. (473) 732 0006 (Ext. 8161)

Adaptive or stationary-phase mutagenesis (SPM) is a process that allows stressed populations of cells to acquire mutations that favor growth after the application of a nonlethal selective pressure1. This cellular process has been successfully studied in the Gram-positive microorganism *Bacillus subtilis* employing the strain YB955, which measures the reversion frequencies of the mutant *hisC952*, *metB25* and *leuC427* genes, under growth-limited conditions[1].

Previous studies reported that suppression or saturation of the guanine oxidized (GO) system (composed by MutM, MutY and YtkD) or the AP-endonucleases Nfo, ExoA, Nth promoted mutagenesis in nutritionally stressed *B. subtilis* cells2,3. Further results revealed that SP mutagenic events promoted by MutY were Mfd-dependent4 suggesting that transcriptional transactions over non-bulky DNA lesions may promote error-prone repair.

The genetic damages that block the progression of the transcriptional and replicative are potentially cytotoxic and genotoxic for cells2. It has long been known that this type of genetic damage is preferably repaired in actively transcribed genes, more specifically in the template transcribed strand, alleviating the cytotoxic effects of these lesions. Based on these observation, here, we inquired whether the transcriptional factors Mfd and/or GreA coordinate the mutagenic events occurring in nutritionally stressed *B. subtilis* cells deficient for GO or the Nfo ExoA Nth repair proteins. To this end, *mfd* and *greA* were genetically inactivated in these genetic backgrounds and the resulting null mutants tested for SPM.

Results revealed that disruption of Mfd or GreA abrogated the production of adaptive His^+ Met^+ and Leu^+ revertants in the GO and *nfo exoA nth* strains, respectively. Taken together, our results support the notion that in nutritionally stressed *B. subtilis* cells, accumulation of spontaneous non-bulky DNA lesions are processed in an error-prone manner with participation of Mfd and GreA.

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UVB and UVC inhibts cellular processes related to carcinogenesis in cervical cancer cell lines

Jesús Adrián López[1], Angelica Judith Granados-López[1], Eduardo Manzanares Acuña[2], Yamilé López Hernández[1], Claudia Araceli Reyes-Estrada[3], Rosalinda Gutiérrez- Hernández[3], Hiram Hernández López[4], Cesar Rivas Santiago[1], José Antonio Varela Silva[1]
[1]Laboratorio de microRNAs, Unidad Académica de Ciencias Biológicas, Universidad Autónoma de Zacatecas. Av. Preparatoria S/N, Zacatecas 98066, México. Tel. 492 156 43 75. Correo electrónico jalopez@uaz.edu.mx.
[2] Unidad Académica de Estudios Nucleares, Universidad Autónoma de Zacatecas. Av. Preparatoria S/N, Zacatecas 98066, México.
[3] Unidad Académica de Medicina Humana y Ciencias de la Salud de la Universidad Autónoma de Zacatecas. Campus Siglo XXI, Kilómetro 6, Ejido la Escondida, CP 98160. Zacatecas, Zac. México.
[4] Unidad Académica de Ciencias Químicas, Universidad Autónoma de Zacatecas. Av. Preparatoria S/N, Zacatecas 98066, México.

The interaction of UV and cell generates free radicals producing cell death, senescence and proliferation alteration among other cell processes changes. UVB and UVC potentially could be used in therapy as an alternative for common radio and chemotherapy. In this work we where interested in seeking the quantity of UV necessary to inhibit cell proliferation and induce cell death. Therefore we make a dose-response and temporal time experiment at different doses of UVB and UVC.

Interestingly we find that UVB and UVC present almost the same effect at a similar energy in SiHa, HeLa, C4.1, C33A and Calo cell proliferation. UVB treatment arrest SiHa cells in S phase in a dose-response shape. Cell migration and invasion shows inhibition since 15 sec of UV exposition showing a profound effect at 25 sec of UV treatment. These UVB cell effects seams protein p53 dependent because it has been shown an increase dependent of UVB doses. These results show that UV can be used for inhibit cellular processes related to carcinogenesis in cervical cancer cell lines opening the possibility to cancer patients.

Effect of folic acid on the regeneration of *Lumbriculus variegatus*

López Martínez Juana María, Arellano-Carbajal Fausto*

Laboratorio de Genética Molecular y Ecología Evolutiva, Unidad de microbiología Básica y Aplicada, Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro. Anillo vial Fray Junípero Serra km 8 s/n, carretera a Chichimequillas, Querétaro, Qro. México, C.P. 76140. Teléfono: (442) 1921200 ext. 65334. Email: fausto.arellano@uaq.mx*

Regeneration is a process whereby organisms can generate lost limbs with the same funcionatily as the previous ones. Annelids have an extraordinary regenerative capacity. One of the members of this phylum is *Lumbriculus variegatus*, an aquatic oligochaeta which has marked anterior and posterior regeneration. Epigenetic changes can promote or suppress gene expression in certain processes. There are multiple compounds that can modify epigenetic patterns, one of them is folic acid or vitamin B9. When folic acid is consumed, it is metabolized to 5-methyltetrahydrofolate, donator of methyl groups for methionine sythesis, which is an essential precursor to cofactor S-adenosylmethionine, involved in methylation reactions. Another function of folic acid is that it intervenes in nucleic acid and amino acid synthesis. It has been described that deficiencies of this compound during development induce alterations in neural tube formation, as well as an increased risk of cardiovascular diseases and carcinogenesis. Therefore, the objective of this work was to evaluate folic acid effect, as a methylating agent, in the anterior and posterior regeneration of *Lumbriculus variegatus*. We aimed to explore if methylation has an important role in this process. For this, we administered 0.05 mg/ml of folic acid and 0.001M NaOH to adult oligochaetes during fourteen consecutive days after being amputated anteriorly and posteriorly. The petri dishes were maintained at 20 °C. The regeneration rate was recorded from day three to day 14 post amputation (dpa). We found that folic acid treatment favored anterior and posterior regeneration between 3 and 10 dpa. Our study is a first approach to understand the role that methylations play in regeneration.

Characterization of *Sporothrix schenckii* strains silenced in the gene encoding for Gp70

López Ramírez, L.A., Luna-Herrera, J. A., Martínez-Álvarez, J. A., Lozoya-Pérez, N. E., Mora-Montes, H. M.*
Departamento de Biología, División de Ciencias Naturales y Exactas, Universidad de Guanajuato, Noria Alta s/n, Col. Noria Alta, Guanajuato, Gto., México. C.P. 36050. *Tel: +5214737320006 Ext. 8193, E-mail: hmora@ugto.mx

S. schenckii is a dimorphic fungal pathogen that causes the Deep-seated mycosis named sporotrichosis, a disease with a significant morbidity in both humans and animals.

The fungal cell wall plays an essential role during the fungus-host interaction, as it confers protection to the fungal cell and works as a molecular scaffold to display virulence effectors. Some *S. schenckii* wall components have been thus far studied, and among them, Gp70 has gained interest due to its adhesion properties and the ability to induce a protective immune response against this pathogen.

Here, to get more insights in the role of Gp70 in the fungal virulence and its participation in the *Sporothrix*-immune system interplay, we generated a construction to silence the encoding gene for Gp70 and used to perform *Agrobacterium tumefaciens*-mediated transformation. So far, we have generated two strains, 1B1 and 4B2, which have 24.76% and 48.91% silencing of this gene, respectively.

To assess the impact of the Gp70 silencing on the fungal cell wall, we conducted assays to measure the ability to bind the cationic dye Alcian Blue; while the cell wall composition was analyzed by HPAEC-PAD. We found a reduced ability to bind the dye and changes in the sugar content in both strains, which were dependent in the silencing degree. Moreover, the virulence of these strains was also affected in the alternative model of *Galleria mellonella*.

Thus far, were conducting more experiments aiming to obtain strains with a higher degree of gene silencing.

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Reconstruction of pantothenate synthesis pathway in rhizobia: where does β-alanine come from?

Mariana López Sámano[1], Tomás Villaseñor Toledo[2], Araceli Dávalos[1], Luis Lozano[1] and Alejandro García de los Santos[1].
[1]Centro de Ciencias Genómicas, UNAM. Av. Universidad s/n Col. Chamilpa 62210, Cuernavaca Morelos, México
[2]Instituto de Biotecnología, UNAM, Cuernavaca 62210, Morelos, Mexico
alex@ccg.unam.mx

Comparative genomic studies have reported numerous cases of missing ORFs in multiple metabolic pathways. One example is the Pantothenate (vitamine B5) synthesis pathway. Pantothenate is the precursor of coenzyme A (CoA) an essential cofactor of a large number of key metabolic enzymes. Pantothenate is synthesized from two precursors: pantoate and β-alanine. In γ-proteobacteria β-alanine is produced in a sole step by decarboxylation of L-aspartate. This reaction is catalyzed by the aspartate decarboxylase enzyme (ADC), encoded by the *panD* gene. Mutants in this gene are β-alanine auxotrophs. Previous bioinformatic studies have suggested the absence of ADC from the genome of members of the order *Rhizobiales.* However, the β-alanine prototrophy of several rhizobios indicates that in the absence of ADC different pathways may be involved in the β-alanine synthesis. We surveyed the presence of 12 proteins involved with the pantothenate synthesis and transport (AmaB, Dht, PyrD, GadB, PanB, PanC, PanD, PanE, PanM, IlvC, alanine aminomutase (AAM) and malonate semialdehyde transaminase) in 204 alpha-proteobacteria. We used Proteinortho v5.15 and Pfam v31.0 database to determine the occurrence of 12 proteins of interest. This analysis revealed that from 83 genomes belonging to members of *Rhizobiales* order, 69 lacks ADC, 12 presented 1 ADC and 2 genomes encode 2 ADC. The enzyme AmaB was found in 56 members of *Rhizobiales*. It is a putative β-ureidopropionase, assumed to be part of the reductive degradation of pyrimidine pathway the main source of β-alanine in mammals and plants. Functional β-ureidopropionase has also been reported in *Clostridium uracilum, Agrobacterium fabrum* C58, *Sinorhizobium meliloti, Pseudomonas* and *Arthrobacter* genera. These enzymes are able to synthesize *in vitro* a variety of β-amino acids with important pharmacological properties. We hypothesized that in bacteria lacking ADC, β-alanine may be synthesized by a β-ureidopropionase. This hypothesis was experimentally tested in *R. etli* CFN42. This bacterium lacks an ADC but its RHE_CH03290 gene encodes a putative AmaB. We demonstrate that its disruption produces a β-alanine auxotrophic mutant that restore its growth by the addition of β-alanine in the culture medium, or by genetic complementation with the RHE_CH03290 wild type gene. We also demonstrated by TLC the presence of β-alanine in the product of the enzymatic reaction.
This study suggests, for the first time, that in bacteria lacking ADC, β-alanine can be sinthesized by a β-ureidopropionase.

Mutations in the RB1 gene of Mexican childrens with retinoblastoma

Autores: Antonio Loreto Velázquez, Mayra Martínez Sánchez, Vanesa Olivares Illana
Instituto de Física —Manuel Sandoval Vallarta," Universidad Autónoma de San Luis Potosí, Álvaro Obregón 64, 78000 San Luis Potosí, SLP, Mexico

Retinoblastoma is a common intraocular malignancy in the world pediatric population being the second most common in children under 4 years age. The incidence of the disease varies depending to the country or even the region. In developed countries the rates are between 2.2. to 6.2 per million habitants; in non-developed countries it can increase up to 24.5 per million habitants. The illness is associated to the inactivation of both alleles of the *RB1* gen (Retinoblastoma gen). Loss of function of RB (Retinoblastoma protein) promotes retinoma by loss of cell-cycle control and can cause genomic instability. That may trigger malignant proliferation by changes in other genes. The purpose of the present work is to characterize the spectrum of germ-line mutations in Mexican patients with retinoblastoma and their possible repercussions at a molecular level, exploring the relationship between the mutation present in RB protein and its function. The importance of this research is due to the lack of information regarding the mutations presents in Mexican population, in order to give useful information for design optimal treatments for the patients and their families, especially because the incidence in this country is higher than the reported worldwide.

Papilloma virus and its potential role in retinoblastoma tumors in Mexican Population

Jesús Adrián Lozano Sánchez1, Mayra Martinez-Sanchez1, Jesús Oria-Hernandez2 and Vanesa Olivares Illana1. 1Laboratorio de interacciones moleculares y cáncer. Instituto de Física, UASLP. 2Instituto Nacional de Pediatría Secretaria de Salud, Distrito Federal, Mexico City, México
vanesa@ifisica.uaslp.mx

Mexico is a country where the incidence of human papilloma virus (HPV) is really high (10,000 cases per year) and also it has one of the first places worldwide of Retinoblastoma (RB) cases registered. HPV it's an infection that has been related with several types of human cancers due to its relation-inhibition on pRB and p53 and how this affects to the control of cell cycle and ending in the development of cancer tumors by the hosts. Several studies have reported an association between HPV infection and retina cancer in Indian and Brazilian population. Working with paraffin-embedded tumor samples and using molecular techniques we are studding if there's any correlation between HPV 16/18 strains virus and patients with Retinoblastoma by evaluating the presence of Papilloma virus DNA on children with retinoblastoma in Mexican population.

Genetic interactions among members of the RNA-directed DNA methylation pathway during reproductive development in Arabidopsis

Judith Lua-Aldama[1] and Jean Philippe Vielle-Calzada[1]
[1]Grupo de Desarrollo Reproductivo y Apomixis Laboratorio Nacional de Genómica para la Biodiversidad. CINVESTAV Km. 9.6. Libramiento Norte. Carretera Irapuato-León. CP36821. Irapuato, Gto. México. +52 (462) 1663000 ext.3064
***vielle@cinvestav.mx**

RNA-directed DNA methylation (RdDM) is the major small RNA-mediated epigenetic pathway that controls de novo genomic methylation, epigenetic reprogramming and eterochromatin formation in flowering plants. It involves the coordinated action of specialized DNA and RNA polymerases, DICER-like and ARGONAUTE proteins, and methyltransferases that drive de novo methylation in CHH, but also CHG or CG contexts (where H= A, T or C). Previous studies in our group have shown that mutants in RdDM proteins show ectopic gametic differentiation and gametophyte formation in the ovule, a phenotype reminiscent of apomixis. Restriction of this phenomenon is under tight and redundant control of proteins of the AGO4 clade. Interestingly, F2 to F4 segregating populations of double mutant combinations involving key members of the RdDM pathway show epigenetic mechanisms of transgenerational inheritance that favor the recovery of specific genotypes at the expense of wild-type individuals. This phenomenon is explained by preferential viability of haploid products following meiosis and not embryo of seed abortion, suggesting that mechanisms of meiotic drive might cause lethality of certain allelic combinations involving wild-type alleles. Our results revealed unforeseen mechanisms of epigenetic inheritance controlling meiosis through the action of small RNA regulatory pathways in Arabidopsis.

Hypermethylation of *PPP2R2B* represents a novel mechanism by which chronic inflammation perpetuates itself.

Iris K. Madera-Salcedo,1 Beatriz Sánchez-Hernández,2 Yevgeniya Svyryd,2 Marcela Esquivel-Velázquez,1 Noé Rodríguez-Rodríguez,1,3 Emmanuel Dávalos-Estela,1 Rogelio Cuervo-Campos,1 Osvaldo M. Mutchinick,2 Jorge Alcocer-Varela,1 Florencia Rosetti,1 and José C. Crispín1*
1 Departamento de Inmunología y Reumatología, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico; 2 Departamento de Genética, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico; 3 Departamento de Inmunología, Instituto de Investigaciones Biomédicas, UNAM, Mexico City, Mexico. *Av. Vasco de Quiroga 15 Col. Belisario Domínguez Secccioón XVI. C.P. 14080 Tlalpan, Cuidad de México. +52 (55) 5487 0900 Ext. 2610. email: carlos.crispina@incmnsz.mx

The B55β regulatory subunit of the phosphatase PP2A, encoded by *PPP2R2B*, controls apoptosis in activated T cells during cytokine withdrawal. In patients with the autoimmune disease systemic lupus erythematosus (SLE), failed expression of B55β is associated with T cell resistance to apoptosis.
The aim of this work was to evaluate the kinetics of expression of B55β in T cells from patients with systemic autoimmune diseases (i.e. SLE, rheumatoid arthritis [RA], and Sjögren's syndrome [SS]) and analyze the genetic and epigenetic mechanisms that regulate its transcription in health and disease.
IL-2 deprivation induced the expression of B55β in T cells from healthy controls, but failed to do so in ~50% of patients with SLE and RA. B55β transcription in response to IL-2 withdrawal was normal in patients with SS. Moreover, apoptosis induced by IL-2 deprivation was significantly impaired in patients with RA and SLE that failed to upregulate B55β. We analyzed the *PPP2R2B* locus, in order to identify regulatory elements that could be linked to the failed transcription of the gene in patients with autoimmunity. First, we focused on a CAG repeat sequence whose expansion has been linked to increased *PPP2R2B* expression. The frequency of repeats was normal in all individuals studied (RA and SLE patients). Next, we analyzed a large CpG island located in a conserved sequence. Using methylation-sensitive PCR, we detected a higher degree of DNA methylation in patients with SLE. We confirmed these finding by pyrosequencing, and in addition, we identified a region that was highly methylated in patients whose T cells failed to transcribe B55β. These results suggested that factors extrinsic to the T cell (inflammatory mediators) could affect DNA methylation at the level of *PPP2R2B*. To test this, we isolated T cells from healthy donors and cultured them in the presence of different cytokines. Compared to T cells expanded in the presence of IL-2, the addition of pro-inflammatory cytokines caused a decrease in the rate of apoptosis and a reciprocal increase in the methylation of *PPP2R2B*.
Collectively, our results have identified a gene whose regulation affects the sensitivity of activated T cells to undergo apoptosis during cytokine withdrawal. Moreover, our data has revealed an epigenetic mechanism through which T cells activated in inflammatory milieus acquire resistance to apoptosis. This may enable them to exert more intense pro-inflammatory activities, but in the context of autoimmunity, it may represent the pathogenic basis of a positive feedback loop that perpetuates inflammation.
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siRNA molecules derived from the dsRNAs synthesized by sense and antisense transcription generate gene silencing in *Giardia lamblia*.

Jaime Marcial-Quino, Saúl Gómez-Manzo, María Fernanda Torres Rojas, Laura Eloísa Morales-Luna, Erick Alcaraz-Carmona Edson Jiovany Ramírez Nava, Víctor Martínez Rosas, Yadira Yazmín Cortés- Morales, Fabiola Fernández Rosario, Horacio Reyes-Vivas.
Instituto Nacional de Pediatría. Laboratorio de Bioquímica Genética, Secretaría de Salud. C.P. 04300. (+52) 55 1084-0900 (ext. 1442). jmarcialqu@conacyt.mx

The microaerophilic protozoan *Giardia lamblia* is the agent causing giardiasis, an intestinal parasitosis of worldwide distribution. Different pharmacotherapies have been employed against giardiasis; however, side effects in the host and reports of drug resistant strains generate the need to develop new strategies that identify novel biological targets for drug design[1]. To support this requirement, we have designed and evaluated a vector containing a cassette for the synthesis of double-stranded RNA (dsRNA), which can silence expression of a target gene through the RNA interference (RNAi) pathway. Small interfering RNAs (siRNAs) were detected and quantified in transformants expressing dsRNA by a stem-loop RT-qPCR approach[2]. In this work, the NADH oxidase (NADHox) gene was used as a model to prove the silencing. The results showed that, in transformants expressing dsRNA of 100-200 base pairs, the level of NADHox mRNA was reduced by around 30%, concomitant with a decrease in enzyme activity and a reduction in the number of trophozoites with respect to the wild type strain, indicating that NADHox is indeed an important enzyme for Giardia viability. These results suggest that it is possible to induce the *G*. *lamblia* RNAi machinery for attenuating the expression of genes encoding proteins of interest as previously reported[3]. We propose that our silencing strategy can be used to identify new potential drug targets, knocking down genes encoding different structural proteins and enzymes from a wide variety of metabolic pathways.

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Transcriptional divergence in homeologous genes *ALT1* and *ALT2* in *Saccharmomyces cerevisiae*

Dariel Márquez and Alicia González
Department of Biochemistry and Structural Biology, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México. P.O. 04510. México. Tel. (+52) 55 56225631; email: dariel.marq@gmail.com

Genetic duplication is one of the main processes to generate new or specialized functions. After gene duplication, divergence may occur at different levels, which leads neofunctionalization or subfunctionalization of duplicated copies. Transcriptional diversification is one of the mechanisms, which due to changes of the *cis* or *trans*-acting elements determine diversification, since these modifications have a strong impact on transcriptional regulation. *ALT1* and *ALT2* are two paralogous genes that originated from the hybridization event, which occurred in the *Saccharomyces cerevisiae* lineage. *ALT1* encodes for an alanine aminotransferase which translocates amino group from alanine to 2-oxoglutarate to form glutamate and pyruvate. Surprisingly, no function has been determined for Alt2 even though these two enzymes share 65% of identity. However *ALT1* is poorly expressed in glucose-ammonium (biosynthetic conditions) and is strongly induced in glucose-alanine (catabolic conditions), meanwhile *ALT2* is only expressed under biosynthetic conditions during first hours, and as the culture grows expression decreases. On alanine as sole nitrogen source, *ALT2* expression is completely repressed. In the present work we have analyzed the transcription factors which are involved in *ALT1* and *ALT2* regulation as well as chromatin organization of both genes in order to determine how nucleosome positioning participates. Analysis of *ALT1* and *ALT2* chromatin organization showed a high correlation with their expression profiles. Subsequently, we studied several nitrogen sources in order to understand their role in the modulation of the transcription and how this expression could change in the presence of glutamine, proline and GABA. Our results show that the quality of the nitrogen source does not *per se* affect transcription, for example proline and GABA are similar in their quality as nitrogen sources, however chromatin organization and expression profiles observed in both genes are completely different: GABA induces *ALT1* and *ALT2*, meanwhile in proline only *ALT1* is active. Suggesting that GABA may have an important role on *ALT2* function.

Two essential genes on the secondary chromosome p42e of *Rhizobium etli* CFN42 participate in cell division

Sofía Martínez-Absalón, Carmen Guadarrama, Araceli Dávalos, Susana Brom and David Romero
Programa de Ingeniería Genómica, Centro de Ciencias Genómicas UNAM Ave. Universidad 2001, Col. Chamilpa. 62210 Cuernavaca, Mor., México. Tel. (777) 3175867; scabsalon@gmail.com

Rhizobium etli CFN42 is a nitrogen-fixing bean symbiont with a genome composed of a chromosome and six large plasmids (p42a-p42f). Plasmid p42e (505 kb) is very stable, being recalcitrant to elimination. A systematic deletion analysis revealed two novel essential genes (RHE_PE00001 and RHE_PE00024) on p42e, validating its designation as secondary chromosome (1). Mutants in these genes cannot be generated unless the wild-type function was provided in trans. RHE-PE00001 is a hypothetical protein with a DUF1612 domain (domain of unknown function) and a helix-turn-helix motif.
RHE_PE00024 is a sensor histidine/kinase hybrid protein, participating in a twocomponent signal pathway. However, the response-regulator protein and the target genes for the signal pathway are as yet unknown. Homologs to both essential genes are restricted to the families Rhizobiaceae, Phylobacteriaceae and Brucellaceae, all of them sharing the characteristic of unipolar growth (2). In this work, we focus on determining the function of the essential genes RHE_PE00001 and RHE_PE00024.
To ascertain the role of these genes, conditional knockdown (cKD) mutants, where expression of the corresponding gene is under the control of a cumate-regulated promoter, were generated. A cKD mutant in gene RHE_PE00024 revealed a striking variation in cell morphology. In the absence of cumate, only 37% of the cells displayed the normal bacillary shape, the remaining 63% is comprised by small, round cells. By DAPI staining, both cell types harbor nucleoids. This phenotype is partially reversed in the presence of cumate (62% of bacillary cells and 38% of round cells). Time-lapse microscopy analyses show that this pleomorphism is maintained in the absence of induction by cumate. Bacillar cells from the cKD mutant have a slower duplication time (3 h, instead of the normal 2 h) while round cells have a duplication time of nearly 6 h.Similar results were obtained with the cKD mutant in gene RHE_PE00001. This mutant in the absence of cumate displayed 25% of small round cells, and 75% of bacilli, both containing a condensed nucleoid. These proportions were unchanged upon cumate addition. These results indicate that both genes participate in the control of cell division and shape. Efforts are underway to determine the specific steps affected, as well as to determine which genes are under control of these possible regulators.

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Effect of ferulic acid on the expression of structural and regulatory genes for the biosynthesis of mycotoxin fumonisin B1 in *Fusarium verticillioides*

Francisco Javier Martínez Fraca, Javier Plasencia de la Parra
Departamento de Bioquímica, Facultad de Química, UNAM. Paseo de la Inv. Científica S/N, 04510, Ciudad Universitaria, CD. MX. (Tel. 56225275), javierp@unam.mx

Maize (*Zea mays L*) is the main cereal grown and consumed in Mexico. Its productivity and quality is affected by the presence of various microbial pathogens. Among those, *Fusarium verticillioides* is the main fungal pathogen isolated from maize. It is the causal agent of seedling blight, as well as of stem-, ear- and kernel-rot, thus it occurs at all stages of plant development. The fungus is a good saprophyte that can survive in soil and crop residue. *F. verticillioides* is able to synthesize various mycotoxins; among them fumonisin B1 (FB1), which is a sphingoid base analog and has several targets on the maize cell, thus it is considered a virulence factor. FB1 synthesis depends on the presence of the *FUM* locus, which contains genes that code enzymes involved in its biosynthesis and transporters that mediate its excretion. Other genes, outside the *FUM* locus, also regulate FB1 synthesis in response to environmental factors. During *F. verticillioides*-maize interaction on the ear, the seed pericarp is the first barrier faced by the fungus before colonization. Ferulic acid is a major component in the maize pericarp and its levels varied between 4.5 mg/g to 26.3 mg/g in different maize genotypes. *In vitro* assays show that this compound inhibited FB1 production and repressed the expression of two *FUM* genes (*FUM1* and *FUM8*). We established an *in planta* model in "Chalqueño" maize, whose seeds contain intermediate levels of ferulic acid (16 mg/g), to study the expression of *FUM1* and *FUM8* genes, and four regulatory genes (*FUM21*, *ZFR1*, *AREA* and *PAC1*) in *F. verticillioides* during early colonization (6 h to 48 h) of maize kernels. Our results show that *FUM1*, *FUM8*, and the regulatory genes *FUM21* and *ZFR1*, were induced early, at 12 h post-inoculation and were associated and was associated with FB1 synthesis. These results suggest that FB1 is produced early during plant-pathogen interaction and supports its role as virulence factor in *F. verticillioides.* This research was supported by DGAPA-PAPIIT project IN213517.

A Phasin protein involved in bioplastic metabolism in *Azospirillum brasilense* Sp7

María de los Ángeles Martínez Martínez, Lucía Soto Urzúa and Luis Javier Martínez Morales.
Centro de Investigaciones en Ciencias Microbiológicas. Instituto de Ciencias. Benemérita Universidad Autónoma de Puebla. Edif. IC-11. Ciudad Universitaria, Col. Jardines de San Manuel. C.P. 72570. Puebla, México. E-mail: luis.martinez@correo.buap.mx

Introduction. Polyhydroxybutyrate (PHB) is a biodegradable and biocompatible compound that exhibits similar characteristics to petrochemical plastics. The Gram negative, α-proteobacteria *Azospirillum brasilense* Sp7 produces high quantities of bioplastic. PHB function as carbon and energy storage compound that favor bacterial competitiveness under starvation conditions. PHB synthesis begins by condensation of two Acetyl-CoA molecules. Then, Acetoacetyl-CoA is reduced to 3-hydroxybutyrate (3HB) and finally, 3HB is polymerized to PHB. The PHB resulting is recovered by granule associated proteins (GAP): synthases, depolymerases, regulators and phasins. Phasins are the major proteins that coat PHB granule and stabilize it, also, regulatory functions as controlling PHB number and size has been assigned. To date, this is the first report of phasin proteins in *A. brasilense* Sp7.
Methods. Proteins containing a Phasin_2 motif (PF0362) in *A. brasilense* Sp7 genome were researched on NCBI, PFAM and SMART databases. Phasin AMK58_RS17065 was deleted. Upstream and downstream flanking regions of AMK58_RS17065 gene were amplified and cloned into pSUP202 generating pSUPAKB. pSUPAKB was movilized into *A. brasilense* Sp7 using *E.coli* S17-1. PHB granules morphology was determined by Transmission Electron Microscopy (TEM). To determinate PHB content, minimal media supplemented with malic acid and ammonia chloride were used as carbon and nitrogen sources. 30, 60 and 90 C/N ratios were evaluated. Also the effect of O2 content was evaluated.
Results and conclusion. TEM images shown a decreased number of PHB granules in mutant strain. PHB analysis shown that the best conditions for improve PHB production are cultivated cells under 90 C/N ratio and subjected to a limited O2 condition. PHB content was altered in *A. brasilense* Sp7 mutant strain and phenotype was restored in complemented strain. Our data support the fact that AMK58_RS17065 gene is a phasin. To our knowledge, this is the first report of phasin proteins in *A. brasilense* genera.

The c-di-GMP protein MucR is necessary for cyst formation but not for alginate synthesis in *Azotobacter vinelandii*

Iliana Chantal Martínez[1], Josefina Guzmán[2], Soledad Moreno[2], Miguel Cocotl[3], Guadalupe Espín[2] & Cinthia E. Núñez[2].

[1] Centro de Ciencias Genómicas Universidad Nacional Autónoma de México
Av. Universidad s/n Col. Chamilpa, Cuernavaca, Morelos. 62210, México
[2]Departamento de Microbiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Av. Universidad 2001, Col. Chamilpa, Cuernavaca, Morelos 62210, México
[3]Departamento de Biología Molecular y Biotecnología, Instituto de investigaciones Biomédicas Universidad Nacional Autónoma de México, tercer circuito exterior, Ciudad Universitaria, CDMX 70228, México.

Contacto: Iliana Chantal Martínez. tel: 52 (777) 514 84 66, email: chantal@ibt.unam.mx

Azotobacter vinelandii is a gamma proteobacteria of the *Pseudomonadaceae* family. During its life cycle it undergoes a differentiation process leading to the formation of cyst resistant to desiccation (Segura *et al.,* 2014). The main components of the mature cyst are the exo-polyssacharide alginate that confers on the drought resistance of the differentiated cell. Alginate is also involved in the formation of biofilms. The second messenger c-di-GMP has emerged as a central regulator in bacteria for the variety of cellular processes under its control. The intracellular levels of c-di-GMP are regulated by the opposing activities of diguanilate-cyclases (DGC) and phosphodiesterases (PDE). In *Pseudomonas aeruginosa* c-di-GMP has been shown to be essential for alginate production (Hay *et al.,* 2009); MucR, an inner membrane protein possessing both DGC and PDE active domains, provides the c-di-GMP necessary for activating the alginate co-polymerase Alg8-44 complex. In the present work we evaluated the role of MucR of *A. vinelandii* in alginate production, biofilm formation and during encystment. We found that, contrary to that observed for *P. aeruginosa*, MucR was not required for alginate synthesis but it was necessary for the initial adhesion and dispersion during biofilm formation. In accordance with these results the swimming motility was negatively affected by MucR. Moreover, MucR was essential for the formation of desiccation resistant cyst in an alginate-independent manner and this process could be regulated by MucR upon epimerarses expression. Finally, our results show that, *mucR* is under control of response regulator AlgR and this regulation is directly by union of its promotor.

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Expression of the small RNAs CrcZ and CrcY regulate Carbon Catabolite Repression in the Nitrogen Fixing Bacterium *Azotobacter vinelandii*

Marcela Martínez Valenzuela, Josefina Guzmán Aparicio, Soledad Moreno, Guadalupe Espín and Cinthia Núñez
Departamento de Microbiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Av. Universidad 2001, Col. Chamilpa, Cuernavaca, Morelos 62210, México.
Contact: Marcela Martínez. Phone: 52 (777) 329 16 29, email: marcelam@ibt.unam.mx

Introduction:
Azotobacter vinelandii, belongs to the *Pseudomonadaceae* family, is a nitrogen-fixing soil bacterium that prefers the use of organic acids than carbohydrates, this preferential use is regulated by Carbon Catabolite Repression (CCR). In *Pseudomonas* spp. Carbon Catabolite Repression (CCR) is exerted by the CbrA/CbrB and Crc-Hfq systems (Rojo *et al*., 2010). Crc and the RNA chaperone Hfq repress the translation of mRNA for the uptake of non-preferred compounds whilst the CbrA/CbrB Two Component System activates the transcription of the sRNAs CrcZ-CrcY which sequester the Crc/Hfq complex thereby allowing growth of the bacterium using the non-preferred substrates (Valentini *et al*., 2014).

Background: Previous study in *A. vinelandii* demonstrated that CbrA/CbrB and Crc-Hfq systems work the same as that observed in *Pseudomonas* spp (Quiroz-Rocha *et al*., 2017). We hypothesized that the expression levels of the sRNAs CrcZ and CrcY modulate the CCR strength during diazotrophic growth whilst the amount of the Crc-Hfq protein complex remains constant under different types of carbon sources. Based on previous reports in this bacteria about the lack of CCR in the presence of fixed N2, we also hypothesized that in the presence of NH4 the expression of the sRNAs increased allowing the simultaneous assimilation of preferred and non- preferred carbon sources.

Results:
The CrcZ and CrcY promoter activity was evaluated using a mixture of acetate and glucose as carbon sources. We found that both promoters increased when glucose is being consumed. Real time qPCR performed with RNA from cells growing exponentially in the mixture of acetate-glucose, confirmed that the number of transcripts during low CCR (glucose) were more abundant than those during high CCR (acetate). In addition, our results clearly indicate the existence of a CCR process even in the presence of a fixed nitrogen source, like NH4. In this condition, the expression of the sRNAs is necessary for the assimilation of less preferred substrates (such as glucose). σ54 drives transcription from *PcrcZ* and *PcrcY* in diazotrophic and non-diazotrophic conditions. Our results show that, Crc levels and hfq mRNA transcripts were constant under both conditions. These results confirm that CCR is modulated by the expression levels of the sRNAs CrcZ and CrcY in *A. vinelandii*.

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Role of ExoA and PolA in *Bacillus subtilis* YwqL-dependent DNA deamination-induced repair.

Jimena Meneses Plascencia[1], Víctor M. Ayala García[1], Mario Pedraza Reyes[1].

[1]Departamento de Biología, Universidad de Guanajuato, Noria Alta, Guanajuato, Guanajuato, CP 36050, México | pedrama@ugto.mx | Tel. (473) 732 0006 (Ext. 8161)

Deamination of DNA bases is one of the most common genetic insults that cells must deal with. The loss of the exocyclic amino group in cytosine lead to the production of uracil. This base analog is a highly mutagenic lesion because its permanence in DNA causes CG to TA transition mutations. The major pathway that protects organisms from adverse effects of uracil is thought to be base excision repair (BER) initiated by uracil DNA glycosylase. However, in *B. subtilis*, uracil is predominantly repaired by the alternative excision repair (AER) pathway, which is initiated by the endonuclease V, called YwqL [1]. Biochemical studies have revealed that YwqL is capable of excisinge hypoxanthine and xanthine from *ds*DNA oligonucleotides, allowing PolA to fill the resulting gap [2]. In the case *ds*DNA substrates carrying uracil or AP sites, YwqL only catalyzed the hydrolysis of the second phosphodiester bond 3´ to the lesions suggesting the existence of additional proteins in this repair subpathway [2]. Epistatic assays pointed to ExoA as a possible candidate to continue the downstream repair events initiated by YwqL [2]. In this work, we evaluated the role of ExoA and PolA in the YwqL-dependent AER pathway during repair of uracil and AP sites from the DNA of *B. subtilis*. Results from a genetic approachs suggested that YwqL, ExoA and PolA work in a common pathway to protect *B. subtilis* from the deleterious effects of uracil. These findings were supported by *in vitro* repair assays, showing that endonuclease activity of YwqL and 3´-5´exonuclease activity of ExoA are enough for removal uracil and AP sites, thus allowing the generation of a gap followed by PolA-dependent repair synthesis. In conclusion, our results describe key components of the alternative repair pathway for the removal of AP sites and deaminated bases in *B. subtilis.*

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Differential expression of components of the hepatic glutamatergic system in cirrhosis and hepatocarcinoma

Isabel Méndez, Ana Cristina García-Gaytán, Isaías Turrubiate, Mauricio Díaz-Muñoz.

Instituto de Neurobiología, UNAM, Campus Juriquilla, Querétaro, México. Blvd. Blvd Juriquilla 3001, campus UNAM-Juriquilla, 76230 Querétaro. Tel (442) 238 1035. isabelcm@unam.mx

Background: Hepatocarcinoma (HCC) is the most common liver neoplasm and in the 85% of the cases is associated with underlying liver inflammatory diseases such as fibrosis and cirrhosis. Identification of molecules responsible for the development of HCC is critical for an early diagnostic and more efficient therapies. Recent studies have demonstrated that the glutamatergic system: glutamate, receptors, enzymes and transporter system, are present in non-neural organs and play an important role in the pathophysiology of certain diseases. In this regard, the metabotropic glutamate receptor type 3 (mGluR3) expression is higher in colon cancer cells and in cirrhotic liver. Besides, an enhanced production of glutamate, that is released by the xC antiporter system, induces autocrine signalling of mGluR3 and promotes the invasive phenotype of breast cancer cells. However, it is not known the expression level of components of the glutamatergic system in the progression of liver pathologies that frequently culminate in HCC. **Methods**: Male Wistar rats received weekly intraperitoneal injections of diethylnitrosamine (DEN- 50 mg/kg body weight) to induce fibrosis (8 weeks), cirrhosis (12 weeks) and HCC (16 weeks). Control rats received saline solution at the same intervals. Hepatic mRNA expression of cytokines, glutamate/cystein xCT antiporter (xCT) and mGluR3 was analyzed by RT-qPCR and mGluR3 protein levels by Western Blot. Intra-hepatic and serum glutamate concentrations were quantified by a commercial kit. **Results**: proinflammatory cytokines and xCT gene expression, and intra-hepatic and serum concentrations of glutamate increased in HCC compared to controls, fibrosis, and cirrhosis. mGluR3 gene expression significantly decreased in cirrhosis and HCC, while relative levels of mGluR3 protein were not modified in any condition. **Conclusions**: Components of the glutamatergic system are differentially modified during the liver injury phases induced by DEN treatment, especially in HCC that could be associated with the pro-inflammatory environment present in the liver pathologies. Enhancement of intra-hepatic glutamate concentration and mRNA expression of xCT suggest an enhanced autocrine signaling of mGluR3 in HCC. Altogether, these results indicate a possible role of components of the glutamatergic system in the onset and development of liver pathologies, such as HCC, and suggest the possibility of using them as markers of liver diseases.

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Parthenogenesis: insights from its molecular mechanisms in plants.

Javier Mendiola, Gloria León-Martínez, and Jean-Philippe Vielle-Calzada
Grupo de Desarrollo Reproductivo y Apomixis. UGA Labotratorio Nacional de Genómica para la Biodiversidad (LANGEBIO). Libramiento Norte Carretera Leon Km 9.6, 36821 Irapuato, Guanajuato. vielle@

Parthenogenesis is the autonomous formation of an embryo without a male genetic contribution. Little is known about the genetic mechanisms involved in this process, and less about the genetic basis and molecular mechanisms that control its induction. In the plant kingdom, three genes have been identified as inductors of parthenogenesis: *BABY BOOM* (*BBM*) is a transcription factor member of the large family of DNA-binding proteins *APETALA 2/ETHYLENE RESPONSE FACTOR (AP2/ERF)*, *BELL* belongs to a family of homeodomain transcription factors widely involved in ovule and gametophyte formation, and *MULTICOPY SUPPRESSOR OF IRA 1 (MSI1)* is a member of the Polycomb group (PcG) of chromatin remodeling complexes acting during early embryogenesis. PcG genes are known to regulate the expression of many genes, including HOMEOBOX genes. We are currently testing the possibility that some of these genes could be involved in the repression of autonomous embryo formation in *Arabidopsis thaliana*. Our strategies are based in the ectopic expression or inactivation of key regulatory factors and CRISPR-based targeted editing strategies in the egg cell or other specific cells of the female gametophyte. These approaches, in combination to phenotypic screens under non-pollinated conditions open new possibilities to attempt the induction of clonal seed formation in a model system. cinvestav.mx

Evaluation of the expression and function of histone methyltransferases in hepatocytes of healthy and obese mice

Paola Berenice Mass Sánchez and Lorena Aguilar Arnal
Instituto de Investigaciones Biomédicas. Universidad Nacional Autónoma de México
Circuito Mario de la Cueva s/n, Coyoacán, Ciudad Universitaria, 04510 Ciudad de México Oficina: 562-28938
Correo: loreaguilararnal@iibiomedicas.unam.mx

Circadian rhythms are cycles with periods close to 24 hours. Sleep/wake cycles, body temperature and the hormone levels are examples of circadian rhythms in mammals. Environmental signaling, through light/dark cycles, is strongly linked to the development of an endogenous clock, which has evolved to adapt physiology, metabolism and behavior into 24 hour periods. The clock can be entrained by different stimuli, such as light, food and physical activity, being the light the most powerful circadian time giver [1,2]. The molecular clock is composed by transcriptional activators or repressors that generate several interlocking feedback loops. Optimal work of the clock machinery sustains metabolic homeostasis in lipids and glucose, but a malfunction in the clock leads to metabolic disorders like obesity and type 2 diabetes [3,4].

Circadian transcription happens within the chromatin context. Histone methyltransferases (HMTs) are epigenetic enzymes which mark the chromatin introducing methyl groups at specific histone residues, rising a ~~-c~~ode" that will be interpreted by effectors. Distinct histone methylations rhythmically appear within genes controlled by the clock (GCC) to assist their cyclic expression [5]. It's been demonstrated that a high fat diet (HFD) alters the transcription of GCC [6,7]. Therefore, it's important to study if there is an alteration of the transcriptional regulation of histone methyltransferases triggered by the exposure to HFD, and its functional implications on CCG expression.

The aim of the project is to evaluate the expression and activity of HMTs in hepatocytes of healthy and obese mice, in order to define epigenetic effectors participating in the obesity phenotype induced by diet.

We selected a set of HMTs based on their expression in mouse liver, previously reported data on their circadian transcriptional profile and their known implication in metabolic processes. C57BL/6 mice were fed with control and HFD diet for 19 weeks; weight gain and insulin and glucose tolerance tests indicated an obesity phenotype. Mice were sacrificed at different circadian times and livers were collected. The results obtained in livers from HFD fed mice show that *Ehmt2* and *Nsd2* HMTs gain circadian oscillation in their transcription depending on the diet. We are particularly interested in the role of the HMT EHMT2, which is involved in metabolic processes such as adipogenesis and cholesterol regulation.

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Identification of germinal and somatic mutations in *BRCA1* and *BRCA2* by next-generation sequencing in patients with breast and ovarian cancer

Oliver Millan-Catalan[1], Miguel Rodríguez-Morales[1], Eduardo López-Urrutia[2], Alma Delia Campos-Parra1, Carlos Pérez-Plasencia[1], [2]
Instituto Nacional de Cancerología, Laboratorio de Genómica. Ciudad de México, México[1]
Universidad Nacional Autónoma de México, FES-Iztacala, UBIMED, Laboratorio de Genómica Funcional. Tlalnepantla, Estado de México[2]
Correspondence author: Instituto Nacional de Cancerología, Laboratorio de Genómica. Av. San Fernando No. 22, Col. Sección XVI, Delegación Tlalpan, C.P. 14080, Ciudad de México, México.
Tel: 5533572955 carlos.pplas@gmail.com

Both, breast and ovarian cancer are heterogeneous diseases with multifactorial etiology; however, in carriers of BRCA1 and BRCA2 mutation, the risk for developing any these types of cancer increases by more than 50%. These genes play a central role in DNA repair mechanisms and cell cycle control. In recent studies it has been demonstrated that germinal or somatic deleterious mutations of BRCA in breast cancer or ovarian cancer patients are associated with a higher overall survival in carriers (48%), with respect to non-carriers (36%); that is, the presence of both BRCA functional genes in a tumor cell, allows it to survive conventional therapy. The aim of this study was to identify *BRCA1* and *BRCA2* germinal and somatic mutations in patients with breast and ovarian cancer. *BRCA1/2* genes were amplified from 25 ng of DNA using Ion Ampliseq BRCA 1 and 2 panel (Thermo Fisher Scientific). We analyzed 126 germinal breast cancer samples, 6.34% were positive for one pathogenic mutation in any of these genes; and we analyzed 46 germinal and somatic ovarian samples, 8.69% of patients carried one pathogenic germinal mutation, we did not identify somatic mutations in this group.
Key words: BRCA1, BRCA2, mutation, ion torrent, breast cancer, ovarian cancer

Analysis of the expression of molecules and transcription factors involved in the signaling pathways NF-κB and Adenylate cyclase in the expression of IL-10 in macrophages

Lourdes Millán Pérez Peña[1], Mariluz Angulo Parra[1,2], Irma Herrera Camacho[1], Nora Hilda Rosas Murrieta[1], Bertha A. León Chávez[3], Palola Maycotte González[4].
[1]Laboratorio de Bioquímica y Biología Molecular, Centro de Química del Instituto de Ciencias, BUAP.
[2]Maestría en Ciencias Químicas, Área de Bioquímica y Biología Molecular, BUAP.
[3]Facultad de Ciencias Químicas, BUAP.
[4]Centro de Investigación Biomédica de Oriente-IMSS.
Edificio IC7, Ciudad Universitaria- Benemérita Universidad Autónoma de Puebla. 72570 Puebla Pue. Tel. 229-5500 ext. 7289. e-mail: loudra50@yahoo.com.mx

SUMMARY

Interleukin 10 (IL-10) is a powerful repressor of proinflammatory cytokine production by almost all cells of the immune system is a key cytokine for the maintenance of cellular homeostasis. Recently, a growing interest in how IL-10 expression is regulated in different immune cells has revealed some of the molecular mechanisms involved in the levels of signal transduction, epigenetics, binding to transcription factors and gene activation. However, the mechanism of transcriptional regulation of IL-10 is not fully understood and it is important to recognize the combination of molecules and transcription factors that regulate its expression for the design of new immune intervention strategies.

In this study, we analyzed the expression of molecules and transcription factors involved in the signaling pathways NF-κB and Adenylate cyclase in the expression of IL-10 in macrophages by qPCR. We observed that in macrophages after a treatment with LPS the expression of IL-10 is not significantly modified, while PGE2 and LPS / PGE2 induce the maximum expression of IL-10 at 4 hours, with an increase of 10 and 30 times, respectively; corroborating the synergistic effect of LPS/PGE2 found by other authors. These results suggest that macrophages acquire a regulatory type phenotype with a high expression of IL-10. On the other hand, the analysis of the mRNA expression of the transcription factors showed that LPS/PGE2 induces the maximum expression at 2 h for c-Jun and at 4 h for STAT3 and p50, with increases of 30, 6 and 10 times, respectively; also that the transcriptional regulation of IL-10 occurs differentially and that it depends on the stimulus and phenotype acquired by macrophages, so that different transcription factors are activated that could be involved in the transcriptional regulation of IL-10.

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Molecular cloning and transient expression of recombinant human PPARγ in HEK293T cells under an inducible Tet-on system

Authors: Sonia Montero Molina, Eder Arredondo Espinoza, Jorge Solis Estrada, Isaias Balderas Renteria. Genetics engineering and Genomics Laboratory, School of Chemistry, Universidad Autonoma de Nuevo Leon.
Av. Universidad S/N, Colonia Universitaria, San Nicolas de los Garza, Nuevo Leon, Mexico.
Telephone: 83294000 ext 3435 and 6221; e-mail: isaias.balderasrn@uanl.edu.mx

Peroxisome proliferator-activated receptor gamma (PPARγ) is involved in the regulation of lipid and glucose homeostasis as well as in cancer and inflammation. PPARγ expression level has been widely studied in multiple tissues, however, there are few reports of preceding attempts to produce full-length human PPARγ (hPPARγ) in cellular models, and generally, expression level is not known or measurable. Here we propose an alternative strategy to express recombinant hPPARγ, using a transient transfection with an inducible and tightly controlled Tet-On 3G system where target and reporter gene were cloned in the same open reading frame. We transiently co-transfected human embryonic kidney 293T (HEK293T) cells with pTRE-ZsGreen1-IRES2-hPPARγ and pCMV-TET3G for inducible expression of hPPARγ after doxycycline administration. Relative expression of the transcript was evaluated 48 hours after transfection by RT-qPCR, obtaining a high expression level of hPPARγ (530-fold change, $P<0.002$) in co-transfected HEK293T cells in presence of doxycycline (1000 ng/mL); also a significantly increased production of the reporter protein ZsGreen1 (3.6-fold change, $P<0.05$) was determined by fluorescence analysis. Therefore, these data indicated that HEK293T cells were successfully co-transfected and it could be an alternative model for hPPARγ expression *in vitro.* Additionally, this model will help to validate the quantification of inducible hPPARγ expression *in vivo* models for future research.

Activity of V2 and V3 promoters from *ST3GAL4* gene under effect of E6 viral oncoprotein from HPV 16

Monterrosas Santamaría José Ricardo[1], Ortiz Mateos Claudia Angelica[1], Cepeda Cornejo Verónica[1], Molina Romero Dalia[1], Venegas Meneses Berenice[1], Vallejo Ruíz Verónica[2], Reyes Leyva Julio[2], *Milflores Flores Lorena[1].
[1]Facultad de Ciencias Biológicas, Benemérita Universidad Autónoma de Puebla, [2] Centro de Investigación Biomédica de Oriente, IMSS, Metepec, Puebla*CP. 72000, 222 2295500 ext. 7076, lorenamilflo@yahoo.com.mx

Introduction: Enzyme ST3Gal4 catalyzes the formation of the NeuAc-alpha-2,3-Gal-beta-1,3-GalNAc- or NeuAc-alpha-2,3-Gal-beta-1,3-GlcNAc-sequences found in terminal carbohydrate groups of glycoproteins, glycolipids, and in the sialyl Lewis X determinant biosynthesis. This enzyme is encoded by ST3GAL4 gene found in chromosome 11q24.2. We know that mRNAs gives rise to six different isoforms A1, A2, V1, V2, V3 and V4, which are produced by alternative splicing and the usage of different promoters. Isoforms type V are found in many cell types, such as: colon, placenta and leukemia, while the isoforms type A, are specifically expressed in testis, ovary and placenta.
The viral oncoprotein E6 from HPV16 has the ability to transform many signaling pathways and contributes to the malignant transformation in the cell participating in the development of cervical cancer through deregulation of genes.
Alterations on the transcriptional regulation of ST3GAL4 gene can change cell cycle behavior, enhancing the metastasis and cancer development. Thus, it would be important to study the transcriptional regulation from promoters V2 and V3 of ST3GAL4 gene and the effect of viral oncoprotein E6 from HPV16 on their transcriptional activity.
Material & Methods: Promoters V2 and V3 were identified from *in silico* analysis, after the promoters were cloned into a vector (pGL4.12) and transfected into HaCat (immortalized human keratinocytes) cell line, then we evaluated the levels of expression from each promoter. Finally, we evaluated the levels of expression from each promoter (V2 and V3) with the expression vector that contains E6 viral oncoprotein promoter from HPV16.
Results: We described a novel promoter called V3, this promoter was discovered by *in silico* analysis and presents high transcriptional activity in a HaCat cell line and showed an increase his level expression when we evaluated together with the E6 oncoprotein, that increase was at least double its activity than normal conditions. For V2 promoter that showed transcriptional activity in a HaCat cell line, when we analyzed the effect of E6 viral oncoprotein we observed a transcriptional activity half-diminished, contrasting with the that reported for a V3 promoter. Conclusions: Transcriptional activity from promoters V2 and V3 were altered by E6 viral oncoprotein showed that E6 contributes to deregulation of this gene and has a role in the cervical cancer development.

Protein composition and biological activities of the venom from *Ophryacus sphenophrys*

Luis Sergio Muñoz Carrillo[1], Mariel Teodora Valdes Arellanes[1,2], Alejandro Alagón Cano[2], Edgar Enrique Neri Castro[2], Bruno Lomonte Vigliotti[3], Rosa Isela ÁlvarezGonzález[1], Eduardo Madrigal Bujaidar[1]

1. Laboratorio de genética, Escuela Nacional de Ciencias Biológicas, IPN
2. Instituto de biotecnología de la Universidad Nacional Autónoma de México
3. Facultad de microbiología, Universidad de Costa Rica

Serpents of the genus *Ophryacus* are endemic of México. *O. sphenophrys* is concentrated in the state of Oaxaca. This genus, as well as its venom is poorly studied. Generally, the venom of snakes is a complex mixture formed by bioactive compounds constituted by proteins and peptidic molecules, including fosfolipases, fosfodiesterases, L-aminoacid diesterases, acetilcolinesterases, metaloproteinases, serinoproteinases, endonucleases, and hialuronidases. Other no enzymatic components such as disintegrins and type C lectins may also be present, as well as some inorganic components. The venom is mainly used to facilitate the immobilization and digestion of the prey, as well as for defense against threats. However, it is also the cause of moderate to severe damage in humans subjected to ophidian accidents.

Considering that no information has been published on *Ophryacus sphenophrys* venom composition, and its biological activities, the present study was designed to fulfill such two aspects. We initially quantified the protein of the venom, and then, applied chromatographic and electrophoretic methods to identify the protein composition; furthermore, we examined various biological effects related with the venom. Our results showed that the venom has a large amount of phospholipase 2, particularly crotoxin, which characterize a neurotoxic activity, the venom showed a high lethal potency (0.8 mg/kg), it has low proteolytic, hemorrhagic and edematizing activity, no coagulant activity and neither cyto/genotoxic effect using the mouse blood micronucleus test. Our results are useful to characterize the venom of *O. sphenoprys.*

Establishment of a cellular model of differentiated human myocytes for the study of the lncRNAs alterations involved in the pathogenesis of myotonic dystrophy type 1 (DM1)

Nadia Mireya Murillo Melo[a,b], Oscar Hernándeza, Bulmaro Cisneros[c], Jonathan J Magañaa,[b]
[a]Laboratory of Genomic Medicine, Department of Genetics, National Rehabilitation Institute (INR), Mexico City, Mexico
[b]Biomedical Sciences Program, Faculty of Medicine, National Autonomous University of Mexico (UNAM), Mexico City, Mexico
[c]Department of Genetics and Molecular Biology, Center of Research and Advanced Studies-National Polytechnic Institute (CINVESTAV-IPN), Mexico City, Mexico

Correspondence: *Jonathan J. Magaña, PhD, Depto. de Genética, Instituto Nacional de Rehabilitación, Calz. México-Xochimilco No. 289, Col. Arenal Guadalupe, 14389 México, D.F., México. Phone: (+52) (55) 59991000, ext. 19403; Fax: (+52) (55) 56039127; E-mail addresses: maganasm@hotmail.com and jmagaña@inr.gob.mx

Myotonic dystrophy type 1 is the most common muscular dystrophy in adults, with a prevalence of 1/8000 worldwide. DM1 is a neuromuscular disorder inherited in an autosomal dominant pattern. It is caused by expanded CTG repeats in the 3' untranslated region (3'UTR) of the dystrophy myotonic protein kinase gene (DMPK). The gene is located on chromosome 19q 13.3. It is a multisystem disorder with a complex pathophysiology. The symptoms and clinical findings include myotonia, muscle wasting, cardiac conduction defects, cataracts and insulin resistance, among others, whereas in the congenital form of DM1 cognitive dysfunction and mental retardation have also been documented. In accordance with the clinical symptoms, the most affected tissue is skeletal muscle, followed by cardiac muscle and smooth muscle, as well as central nervous system (CNS). Due to the importance of skeletal muscle, the characterization of the mechanism involved at the muscular level has been a research priority.
In this study we create a cellular model based on primary cultures of human fibroblasts that have alleles from the DMPK with a normal number of CTG repeats (<30 repeats) or alleles with a number of CTG triplets in the mutant range (1000 and 1700 repeats), obtained from control subjects or patients with DM1, respectively. DM1 fibroblasts were purchased from Coriell Institute. We performed PCR and TP-PCR (Triple Primer PCR), which was then analyzed by capillar electrophoresis to evaluate the expansion of repeats. An electrophoretic pattern was identified that corresponded to the mutation in DM1 fibroblasts, and the number of repeats was corroborated by SP-PCR (defined by Southern blot). A FISH analysis was performed to observe the presence of nuclear foci characteristic of DM1. Once the cultures were characterized, the fibroblasts were induced into muscular differentiation through stable transfection (nucleofection) with a vector that codes for the *MyoD* (pQBI-MyoD) gene, the principal gene that controls differentiation of muscle tissue. The fibroblasts presented morphological changes with the presence of elongated and multinucleated cells compared to control cultures and a difference in the fusogenic index. We examined MyoD expression by RT-PCR in DM1 cultures and controls 18 days post-transfection. Marker expression of MyoD muscular diffentiation was also evaluated with immunofluorescence, where the expression of these markers corresponded to morphological myocyte cells. We verified the DM1 characteristics in patient fibroblasts and the phenotype of the fibroblasts post-transfection suggests the differentiation toward muscular tissue, which provides us with a model very close to the DM1 patient tissue.
This model allows us to determine the profile of lncRNAs in the DM1 associated with the transcriptome alteration and their involvement in the pathogenesis of this disease. We will evaluate lncRNAs and alternative splicing modifications, as well as the deregulation of other transcripts in order to determine their relationship, to in turn determine their key role in the disease.

TGF-β1 induced profound methylation changes in fibroblasts, and revealed an unexpected role of Homer1 in idiopathic pulmonary fibrosis

Miguel Negreros, James Hagood, Celia Espinoza Yalbi Balderas, Moisés Selman. Annie Pardo
Facultad de Ciencias, UNAM
Cto. Exterior s/n, Ciudad Universitaria, 04510 Ciudad de México, CDMX
apardos@unam.mx tel. 56233822

RATIONALE Idiopathic pulmonary fibrosis (IPF) is a complex disease of unknown etiology. Environmental factors can affect disease susceptibility via epigenetic effects. There are few studies that explore global DNA methylation in IPF lungs fibroblast and none focused on Transforming Growth Factor beta-1 (TGF-β1) as a potential modifier of methylome. Here we analyzed normal and IPF methylome and transcriptome before and after TGF-β1 treatment, and one of the modified genes: Homer1. As far as we know this is the first study that explores the potential role of Homer1 in IPF.

METHODS One Primary culture of fibroblasts derived from normal human lung and one from IPF were treated with TGF-β1 (10ng/ml) for 24 h and 5 days. mRNA expression was analyzed with GeneChip PrimeView Human Gene Expression Array (Affymetrix) and DNA methylation status with the Infinium HumanMethylation450 BeadChip Kit (Illumina). Expression of Homer1 was validated with RT-PCR, and confirmed at the protein level with Western blot. Lung localization of Homer1 was examined by immunohistochemistry. Inverse transfection of siRNA against Homer1 in normal fibroblasts was performed. Apoptosis was analyzed with FLICA assay and TUNEL. Intracellular calcium release was examined with Fura-2 AM assay. Proliferation was measured with WST assay.

RESULTS We analyzed at single nucleotide resolution the DNA methylome not only at promoters but also at other gene regions (gene body, 3' and 5' UTRs, 1st exon, between others) and also CpG Island reference: shore, shelf, open sea or island itself. We found that IPF fibroblasts with TGF-β1 treatment showed more methylation changes versus normal fibroblasts. Surprisingly we found that treatment with TGF-β1 in IPF fibroblasts resulted in hypermethylation but upregulation of Homer1, a scaffolding protein that has been implicated in Calcium modulation signaling. We confirmed the over expression of the gene and protein after TGF-β1 long treatment (5 days) in fibroblasts. Silencing of Homer1 significantly increased cell death, intracellular calcium release and decreased proliferation. Interestingly Homer1 was immunolocalyzed in fibroblastic foci in IPF lungs.

CONCLUSIONS Our findings demonstrate that TGF-β1 treatment modifies methylation patterns in IPF. Homer1 a hypermethylated and upregulated localized in fibroblastic foci in IPF lungs might play a role increasing resistance to apoptosis.

Alteration in the proliferation and migration of colorectal cancer cells derived from the interaction of miR-124 and GPT2.

David Núñez Corona, Jossimar Coronel Hernández, Eduardo López Urrutia Verónica García Castillo, Carlos Pérez-Plasencia.
Laboratorio de Genómica Funcional del Cáncer L-11 ubicado en Unidad de Investigación en Biomedicina (UBIMED). Avenida de los Barrios Número 1, Colonia Los Reyes Iztacala Tlalnepantla, Estado de México, C.P. 54090.
Teléfono 56 23 13 33 Ext. 39807 carlos.pplas@gmail.com

Summary
Cancer cells are distinguished from most normal cells by their re-programmed metabolism. These cells convert glucose into lactate even in the presence of oxygen (warbug effect) and use glutamine to generate metabolic intermediates that complete the tricarboxylic acid (TCA) cycle. For glutamine to take advantage of TCA, it has to undergo a deamination process mediated by glutaminases such as glutamate pyruvate transaminase 2 (GPT2), resulting in the TCA intermediate α-ketoglutarate (α-KG); it has been reported that GPT2 overexpression enhances glutaminolysis and facilitates cell migration and proliferation. miR-124 is a small non-coding tumor suppressor molecule downregulated in colon cancer cells, but with an unknown role. Through bioinformatic analysis, we identified mir-124 as a potential GPT2 regulator. We measured the GPT2 mRNA levels in colon cancer-derived cell lines HCT 116, SW480, and SW620, and we found that GPT2 expression was upregulated in the SW620 cell line. In addition, we measured the expression of miR-124 in the same cell lines. We found the relationship between mir-124 and GPT2 trough luciferase assay and intend to further evaluate the role of GPT2 plays in colorectal cancer metabolism.

Debaryomyces hansenii alternate CUG codon analysis

Daniel Ochoa Gutiérrez, Viviana Escobar Sánchez, Claudia Segal Kischinevzky.

Laboratorio de Biología Molecular y Genómica, Edificio A, 2do piso, Facultad de Ciencias, UNAM. Cto. Exterior s/n, Ciudad Universitaria, 04510 Ciudad de México, CDMX
56-22-48-31 biolibre@gmail.com, vv.escobar@gmail.com, claudia.segal@gmail.com

Debaryomyces hansenii is a non-conventional yeast from *Saccharomycetaceae* family. *D. hansenii* is an osmo-, halo-, xerotolerant, psychrophilic, oleogenic, non-pathogenic yeast and is important in a widespread of biotechnological process. It grows at 0.6 M NaCl and it can be cultivated in media with up to 4 M NaCl. The species can survive a pH range between 3 and 10. Moreover, *D. hansenii* has been included in the CTG-clade. In this group of yeasts, CUG codon can be ambiguously translated mainly as serine but also as leucine. This allows them to have a hypothetical proteome that increase for any CTG codon in the gene sequence and rising the phenotypic variability and adaptation to the environment.

In *Candida albicans*, which is also a CTG-clade species, it has been discovered that the leucine tRNA with the CAG anticodon ($tRNA_{CAG}^{Leu}$) is an hybrid and has sequence modifications that enable the seryl-tRNA synthetase (SerRS) and the leucyl-tRNA synthetase (LeuRS) to recognize it and bind a serine or a lucine in the tRNA, having two forms of the charged tRNA ($tRNA_{CAG}^{Ser}$ and $tRNA_{CAG}^{Leu}$). After affinity analysis between LeuRS and SerRS to the tRNA it has been demonstrated that *in vitro* there is no preference for any aminoacyl-tRNA synthetase, but *in vivo* this process occurs 97% of the time for serine and only 3% for leucine. In this fungus, the manipulation of CUG mistranslation (up to 28% of leucine incorporation at CUG decoding) triggered virulence factor expression, such as adhesion, phenotypic switching, morphogenesis and extracellular hydrolase production. All this data suggest an important regulation system of the $tRNA_{CAG}$ and both aminoacyl-tRNA synthetases and maybe, all this process, could be involved in adaptive process for yeast survival.

There are no previous studies about this process in *D. hansenii* but the $tRNA_{CAG}$ and aminacyl-tRNA synthetases gene and protein sequences suggest that there is a similar but completely different process. In this project, the proposal is to understand the alternate codon usage of this yeast by quantitative expression analysis and gene constructions in a heterologous system to analyze differential regulation of $tRNA_{CAG}$ and LeuRS and SerRS genes and see how this differences can provide adaptations to the extreme environments where *D. hansenii* usually live and growth.

We have been working with different media NaCl concentration and pH values to find the conditions where *D. hansenii* has colony phenotype, viability and growth changes. With this, we expect to analyze by quantitative methods the transcription rates to detect different gene regulation levels. This differential regulation values in the aminoacyl-tRNA synthetases would suggest that the transcriptional regulation is important for the capability of $tRNA_{CAG}$ to be bind with serine or leucine, more over than the proteins affinity as it has been suggested previously.

Interaction of RE1-Silencing Transcription factor with E6 gene of the Human Papillomavirus: a plausible mechanism for E6 transcription regulation

Carlos Ortuño Pineda1*, Rabit Jesus Jaimes Bustamante1, Marco Antonio Flores Dircio1, Jesús Valdés Flores2, Napoleón Navarro Tito1 y Miguel Ángel Mendoza Catalán1
1 Universidad Autónoma de Guerrero. Facultad de Ciencias Químico Biológicas. Av. Lazaro Cardenas s/n. Col. La Haciendita, CP. 39087
2 Centro de Investigación y de Estudios Avanzados del IPN. Departamento de Bioquímica. Av. IPN s/n, Col. San Pedro Zacatenco. Ciudad de Mexico, CP. 07360
*Corresponding author: Dr. C. Ortuño. E-mail: carlos2pineda@hotmail.com; Cel 5531543508.

Abstract

The Human Papillomavirus (HPV) infection have been shown to cause cervical cancer (CC) because they are able to transform cells in malignant tumors. After viral invasion, the progression from premalignant lesions to cancer of the cervix depend of viral oncoprotein E6 levels, which modify the p53 expression and cell cycle. The high risk HPV (hr-HPV) express abundant E6 protein compared with low risk HPV (lr-HPV) and it is a key event during cervical carcinogenesis. However, regulation of E6 expression at transcriptional level is not clear. Currently, our group identified the Restrictive Element 1 (RE1) in the HPV16 genome (a hr-HPV) near to the E6 gene. RE1 sequence is the consensus for the RE1 Silencing Transcription factor (REST) interaction, favoring assembly of a repressor complex, thus suggesting a role for REST in E6 expression regulation. The goal of this work was to identify putative RE1 sites across the VPH16 genome and to evaluate the REST-E6 gene interaction by means of Electrophoretic Mobility Shift Assays (EMSA), using recombinant REST and synthetic probes containing the RE1 sequence of VPH16 and a RE1 sequence from HPV11 (an lr-HPV). We bioinformatically identified the RE1 site in E6 gene of HPV16 and designed probes for binding experiments. The dissociation constants were determined from EMSA data, using the equation Kd= [RE1][REST]/[RE1-REST]. Results showed that RE1 site in E6 gene is conserved when compared with RE1 consensus (score = 60%). The RE1 site in HPV16 genome showed a dissociation constant of 4.0 x10-8 , whereas dissociation constant for the RE1 site in HPV11 genome was 4.8x10-8 . In our interpretation, discrete differences in dissociation constants between HPV16 and HPV11 RE1 sites, could explain the transcription rate of E6 gene. This preliminary data constitute the first evidence in E6 regulation mediated by REST.

Key words: Oncoprotein E6, RE1 sequence, dissociation constant.

Analysis of the interaction of YwqL and MutSL during the processing of deaminated DNA bases in *Bacillus subtilis*

Adriana Guadalupe Patlán-Vázquez, Victor Ayala-García, Mario Pedraza-Reyes[1].
Departamento de Biología, Universidad de Guanajuato, Noria Alta, Guanajuato, Guanajuato, CP 36050, México | pedrama@ugto.mx; adrypatlan@hotmail.com | Tel. (473) 732 0006 (Ext. 8161)

Intracellular and environmental factors promote the loss of exocyclic amino groups from the DNA bases cytosine, adenine and guanine generating the base analogs uracil, hypoxanthine and xanthine, respectively. These pre-mutagenic lesions are potentially lethal, and need be repaired. *B. subtilis* possesses a unique uracil DNA glycosylase-encoding gene termed *ung*. Ung eliminates uracil from DNA employing components of the canonical Bases Excision Repair pathway (BER); however, its genetic inactivation only promoted an unexpected mild mutagenic phenotype in *B. subtilis*[1]. Further genetic studies revealed that *ywqL*, encoding a type Endo-V endonuclease, plays a more prominent role in counteracting the mutagenic effects of base deamination in this microorganism1. Further studies have revealed that the MMR system of yeast is able to recognize U/G mispairs[2].

Sequence-specific DNA methylation of old DNA strands is employed by the *E. coli* MMR system (MutS, MutL, MutH) to identify a mispaired base during *de novo* synthesis of DNA[3]. However, most bacteria, including *B. subtilis*, lack a mechanism of DNA methylation; thus the signal that direct the MMR (MutS, MutL) in this bacteria to carry out the correct processing of DNA mispairs is currently a matter of discussion. Current hypothesis point to the existence of single strand breaks during processing of Okazaki fragments in the lagging strand and the incorporation of uracils in the leading strand2. Therefore, the present work is aimed to investigate whether EndoV-dependent endonucleolytic events provide a signal for the MMR to correctly processing mispaired bases in the leading strand. To commence to explore this notion, we employed a genetic approach; to this end, we constructed and characterized a collection of null mutants of *B. subtilis* with the following phenotypes, *ung mutSL*, *ywqL mutSL* and *ung ywqL mutSL*. The experiments with these strains that analyzed its susceptibility to DNA-deaminating agents strongly suggested that Ung and YwqL act cooperatively with MutSL to counteract the cytotoxic and genotoxic effects of DNA deamination. Current experiments investigate the mechanism that contributes to DNA deamination to provide a signal for the correct operation of the MMR system in *B. subtilis.*

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The role of miRNA regulation during sarcopenia in skeletal muscle from aged female rats.

Gibrán Pedraza-Vázquez[1,2,3,4], Beatriz Mena-Montes[1,2,3], David Hernández-Álvarez[1,2], Arturo Simoni-Nieves[1,2,4], Juan Carlos Gómez-Verján[3], Mina Konigsberg-Fainstein[2], Luis Enrique Gómez-Quiróz[2,4], Armando Luna-López[3*].

[1]Posgrado en Biología Experimental, UAMI. [2]Depto. Ciencias de la Salud Universidad Autónoma Metropolitana Iztapalapa. [3]Depto. Investigación Básica, Instituto Nacional de Geriatría. 4Unidad de Medicina Translacional, Instituto Nacional de Cardiología –Ignacio Chávez". *Blvd. Adolfo Ruiz Cortines (Periférico Sur) 2767, Col. San Jerónimo Lídice, Del. La Magdalena Contreras, Ciudad de México C.P. 10200, email: allbioexp@yahoo.com

Loss of skeletal muscle mass during aging, also known as sarcopenia, is a health problem within Mexican population because the proportion of elderly people has increased in the last years. The etiology of sarcopenia remains unknown, however, recent studies revealed that sedentarism, fat gain and genetic regulators such as miRNAs are key regulators in the development of this phenomenon. Aerobic exercise has been recognized as the most effective treatment to prevent and treat sarcopenia. In this work, an experimental model of sarcopenia has been established by keeping groups of rats in conditions of sedentarism and other groups under a low intensity exercise routine. The degree of sarcopenia was determined by DXA (Dual-energy X-ray Absorptiometry) at different age stages and RNA extracted from gastrocnemius muscle was used to perform miRNA microarrays to evaluate the differential expression during lifespan.

Our results showed that sedentary rats gained over 25% of fat with aging; muscle mass diminished almost 20% in 16 months old rats. Our data point towards that age as the start point of sarcopenia. The exercise group did not show an important decrease in muscle mass but displayed a better performance. MiRNA microarrays also showed that a set of miRNAs changed their expression with exercise, these miRNAS are linked to processes like differentiation, cell death and survival. In conclusion, our data suggests that sedentary rats develop sarcopenia at 16 months old, which was delayed in the exercised rats group at that early stage. We also identified a set of miRNAs associated to the progression of this phenomenon that are set to be part of further investigations.

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Transcriptome analyses of miR-122 knockdown breast cancer cells reveal new insights into molecular determinants of resistance to radiotherapy

Isidro Xavier Pérez Añorve[1,2], Claudia Haydée González de la Rosa[2], Ernesto Soto-Reyes[2], Fredy Omar Beltrán Anaya[3], Marisol Salgado Albarrán[2], Oscar del Moral Hernández[4], Elena Aréchaga Ocampo[2*].
[1]Natural Sciences and Engineering Postgraduate, Metropolitan Autonomous University, Cuajimalpa Unit; [2]Department of Natural Science, Metropolitan Autonomous University, Cuajimalpa Unit; National Institute of Genomic Medicine[3]; Autonomous University of Guerrero4. Av. Vasco de Quiroga 4871, Cuajimalpa de Morelos, Santa Fe Cuajimalpa, Postal Code: 05348, Mexico City.
*earechaga@correo.cua.uam.mx

MicroRNAs (mRNAs) play an important role in cancer, some of them has been related with resistance of several therapies including radiotherapy. In particular, mir-122 has been associated with tumor suppressor gene function in breast cancer. However, its role in resistance to radiotherapy is still unknown. In our laboratory we developed a radioresistance model of breast cancer cells (MCF-7RR and MDA-MB-231RR) in which we observed that miR-122 was up-regulated. Here we focused to uncover the molecular mechanism of miR-122 in resistance to radiotherapy in breast cancer. In order to address our question, we use a radioresistance model of breast cancer cells (MCF-7RR and MDA-MB-231RR) were miR-122 is up-regulated. Using an antagomir-122 in MCF-7RR cell line we knockdown (KD) the expression of miR-122, then we analyzed their transcriptome by microarrays. We found a group of 158 genes, of which 27 were up-regulated and 131 down-regulated. By in silico analysis we identify 39 genes (9 up-regulated and 30 down-regulated) with miR-122 binding sites. The analysis of the topological structure of the protein-protein interactions network was performed using the Network Analyzer plugin Cytoscape 3.5.1. Our analysis showed genes such as MOV10, APP, ELAVL1, SIRT7, EWSR4 and EGFR that we did not observed dysregulated in transcriptoma. This suggests that these genes could be implicated in molecular process related to radioresistance. The gene ontology and biological pathways revealed that genes modulated by KD of miR-122 might have a role in processes related to transcriptional regulation, G-protein coupled receptor signaling pathway, TNF pathway, Ras-MAPK pathway and inflammatory response. Also, we validate the expression of a set of genes (ZNF611, ZNF304, RIPK1, and DUSP8) modulated by the loss of function of miR-122 in both radioresistance cells (MCF-7RR and MDA-MB-231RR). Our results may suggest that miR-122 can control the expression of genes related to survival, maintaining a radioresistant phenotype in breast cancer cells. These data open new insights into using mir-122 as a possible marker in cancer.

Role of ZNF365 in Pulmonary Fibrosis

Juan Manuel Pérez Huacuja Urista[1], Mariel Maldonado[2], María de los Remedios Ramírez[1],Carina Becerril[2], Moisés Selman[2], Annie Pardo[1].

[1]Facultad de Ciencias, Universidad Nacional Autónoma de México, CDMX.
[2]Instituto Nacional de Enfermedades Respiratorias, —Ismael Cosío Villegas", CDMX.
Av. Universidad 3000, Circuito Exterior S/N Delegación Coyoacán, C.P. 04510 Ciudad Universitaria, D.F. México. Tel. 56224800. Ext. 84075 y 84076. Email. apardos@unam.mx.

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, irreversible and lethal disease of unknown etiology. Actual hypothesis pose that there is an unknown damage at alveolar epithelial cells, which in turn become aberrantly activated and start with the synthesis and secretion of several cytokines and growth factors, between them TGF beta is one of the main orchestrators of fibrotic processes. TGF beta provokes the activation, migration and proliferation of fibroblasts residing in the lung which differentiate into a more aggressive cell type known as myofibroblast. *ZNF365* is a zinc finger protein that belongs to the C2H2 domain family and has been poorly described in the literature, but it has been seen implicated in cell cycle regulation, DNA double strand break resolution, telomere stabilization and in neural migration in early embryonic mice. Upregulation of *ZNF365* gene has been reported in normal lung and IPF derived fibroblasts stimulated with TGF beta. The purpose of the study was to evaluate *ZNF365* expression in IPF lung tissue and in experimental bleomycin induced lung fibrosis, as well as some functional effects on lung fibroblasts and epithelial cells in vitro by overexpressing and downregulating it in the mentioned cell types.
Expression of *ZNF365* and *Zfp365* mRNA and protein were evaluated by qPCR and WB respectively. For the evaluation of the location and expression between IPF patients and control donors, and between bleomycin instilled mice and saline controls inmunohistochemical analysis was performed. For silencing experiments, specific siRNAs and scrambled controls designed for *ZNF365* were used. Silencing of mRNA and protein was validated by qPCR and WB respectively, and growth rate experiments were performed using WST-1 reagent.
We found that *ZNF365* is over expressed in IPF lung tissues and localized in alveolar and bronchial epithelial cells and in fibroblasts/myofibroblasts. In the mouse model, the orthologous *Zfp365* is overexpressed in lung tissue from bleomycin instilled mice, and the immunoreactive protein was observed in alveolar and airway epithelium. In vitro studies revealed that *ZNF365* is overexpressed in control and IPF fibroblasts under the effect of TGF beta at 24h and 5 days of stimulus. Likewise, TGF beta induced the upregulation of *ZNF365* in A549 alveolar epithelial cells. Preliminary experiments showed that *ZNF365* silencing in A549 alveolar epithelial cells induced a significant reduction of growth rate at three different times of evaluation (24h, 48h and 72h) compared with the scrambled and WT controls ($p<0.05$).

These results demonstrate that *ZNF365* is upregulated in human and experimental lung fibrosis and may be involved in cell proliferation.

Transformation of *Metarhizium guizhouense* mediated by *Agrobacterium tumefaciens*

Iván Horacio Piña-Torres 1*, Arelí Durón-Castellanos 1, Juan Carlos Torres-Guzmán 1, Gloria Angelica González-Hernández1, Israel Enrique Padilla-Guerrero 1**.
1 Department of Biology, Division of Natural and Exact Sciences, University of Guanajuato, Campus Guanajuato, CP 36050, Guanajuato, Mexico.
email: * ih.pinatorres@gmail.com, ** ie.padillaguerrero@ugto.mx

Fungi of the *Metarhizium* genus are a versatile study model based on their biotechnological applications, which have been implemented for over 100 years. Aside from insects, these fungi have been demonstrated to be safely compatible with other organisms (plants, animals, and humans) due to their entomopathogenic status.
Recently, the ecological impact of members of the *Metarhizium* genus and their potential as biological control agents has been reinforced by reports that *Metarhizium* fungi perform more environmental functions than previously believed. For example, *Metarhizium* fungi can interact with plants by colonizing their roots in a mycorrhizal and endophytic manner, establishing a symbiotic relationship with the plants, which stimulates their root growth, activates their defense system and increases their resistance to abiotic factors, such as salinity. *Metarhizium* fungi also transfer nutrients, such as nitrogen, that are obtained from insect fungal parasites from the soil to the plant roots. In turn, plants transfer carbon compounds to the fungi, indicating that *Metarhizium* fungi and plants have a mutualistic symbiotic relationship.
To establish a transformation system in *M. guizhouense* using *Agrobacterium tumefaciens*, we evaluated the antibiotic phosphinothricin and nourseothricin. Subsequently, two vectors containing the phosphinothricin and nourseothricin resistance cassettes whit the fluorescents proteins mCherry and eGFP expression cassettes, respectively, were introduced independently into *A. tumefaciens* and used to transform *M. guizhouense* either in independent events or in one co-transformation. This method provides a genetic transformation system for *M. guizhouense*.

Finding insights on telomere dynamics in telomerase negative strains of *Ustilago maydis.*

Carmen María Posadas Gutiérrez[1], Candelario Vázquez Cruz[2], Estela Anastacio Marcelino[2], María Patricia Sánchez Alonso[2]
[1]Posgrado en Microbiología, Centro de Investigaciones en Ciencias Microbiológicas. [2]Centro de Investigaciones en Ciencias Microbiológicas. [1,2]Instituto de Ciencias de la Benemérita Universidad Autónoma de Puebla. Av. San Claudio y 24 sur, C.U. San Manuel, 72570. Puebla, Pue. Tel. +52 222 229 55 00 ext. 2534 E-mail: maria.sanchez@correo.buap.mx

Ustilago maydis the basidiomycete that causes the corn smut has provided an excellent model system for plant pathogenicity, DNA recombination and repair, cellular signaling, transcriptional and post-transcriptional regulation, cell cycle control, as well as telomere analysis. This last issue has been addressed by our working team with the isolation and characterization of the *trt1* gene, which encodes for the catalytic subunit of the telomerase of *U. maydis.* Telomerase-negative strains, then constructed by one-step disruption, showed reduced lifespan, delayed replication, aged colonial and cellular morphology, and the arising of survivors after 180 replication rounds. Telomere function was partially recovered in survivors by recombination-based amplification of telomere repeats (Type II-like), or subtelomeric *UTASa* plus few telomere repeats (Type I-like).

However, *UTASa* hasn't the same structure as Y' in *S. cerevisiae,* and moreover, a third subelomeric sequence, named *rumT* is also over-amplified in *trt-* mutants. To gain insights on the identity of the amplified segments of *UTASa*, in this project we have established two approaches: (a) characterization of the over-amplified fragments in the Type I-like survivors, b) to disclose the electrophoretic karyotype of the Type I-like mutants measuring its stability over several replication rounds. For this work we used the strains 521 (*a1, b1*), *trt1-1* a Type I-like strain derived from 521, *trt1-2* a Type II-like strain, as well as two wild type isolates which harbor few copies of *UTASa* subtelomeric repeat. For the first objective four gel bands of 3.0 kb, 1.6 kb, 0.65 kb and 0.3 kb containing over amplified DNA fragments hybridizing the *Pst*I-TRF was excised; DNA were extracted by Freeze-Squeeze and cloned in pBluescript KS-. Cloned fragments sent to sequencing. DNA fragments from 3.0 kb and 1.6 kb harbored the seven-motifs helicase domain of SF2 helicases, in addition to *rumT.* Electrophoretic karyotype was obtained by PFGE in CHEF DRII (BIO-RAD); conditions varied according to the resolution purposes. We found significant differences in the electrophoretic karyotype of both types of survivors, mainly increases in chromosomes size of around 50 kb, easy visible in chromosomes 21, 22 and 23; karyotype pattern, however, is still distinguishable. This is congruent with the long digestion times required for *Bal*31 to remove telomere or subtelomeric repeats in *trt1-* mutants. Embedded DNA was digested with *Pst*I and subjected to PFGE to discern the length of the telomere or subtelomere repeats. Hybridization patterns are shown and discussed for characteristics unique in this microorganism. Besides, report nuclei analysis of parental and mutant strains by fluorescence microscopy using IP to correlate mutant phenotype of the nuclei with survivor type to better understanding of the behavior of chromosome ends in absence of the telomerase enzyme.

Fungal metabolities as putative epi-modulators: A case study with BET bromodomain BRD4

Fernando D. Prieto-Martínez*, Mario Figueroa, Nicholas H. Oberlies, Cedric J. Pearce, José L. Medina-Franco.
Departamento de Farmacia, Facultad de Química. Universidad Nacional Autónoma de México.
Avenida Universidad 3000, Deleg Coyoacán, C.P. 04510, Ciudad de México.
*ferdpm4@hotmail.com

Epigenetics involves the chemical modification of the sidechains in the histone octamer. Said modifications are made by enzymes classified as writers, erasers and readers. Previous research has stablished the potential of the epigenome as the missing link in the biogenesis of disease, such as cancer or even Alzheimer's. Because of this, several epi-modifications have been studied, acetylation being a prominent example.

Bromodomains are epigenetic readers related to the expression/suppression cycle, classified on eight families comprising more than 60 isoforms. It has been shown that these readers have direct impact on the development of certain types of cancer, inflammation and diabetes.

Due to this, many researchers have turned their attention to the discovery and development of bromodomain inhibitors as novel therapies. However, it has been suggested that such developments have reached an impasse or current knowledge cannot justify clinical usage of such therapies.

Hence, current efforts should focus on the identification and optimization of probe molecules and novel scaffolds to identify the structure-activity relationships for epi-enzymes. On this regard, natural products have always served as prime source for drug discovery. On the epigenetic landscape, romidepsin is one of the few approved drugs for the treatment of lymphoma by epi-modulation and is a tetrapeptide first identified on bacteria.

Herein we present a virtual screening study, based on fungal metabolites with previous reports of drug-likeness. Searching for novel scaffolds for bromodomain inhibition, using a previously developed protocol. Said protocol consisted on similarity searches using 2D and 3D representations for comparison against 14 known inhibitors of BRD4. Selected compounds were filtered further using consensus docking with four programs: LeDock, MOE, PLANTS and Vina.

Using this method 12 *hits* were found, which were compared with a pharmacophoric template. Yielding only one putative ligand: cephalochromine (a bis-naphto-γ-pyrone). This compound was evaluated by means of Alpha Screening against the BRD4 tandem (BD1+BD2), showing an IC_{50} in the micromolar range (1 μM). To further assess the putative binding mode of this compound, molecular dynamics (MD) simulations were conducted. MD showed that cephalochromin makes two sustained contacts with N140 and a water bridge with D145, both interactions have been described as significant in previous studies.

In summary, we believe that this work shows the great potential of the described protocol while also serving as a proof of concept for the chemoinformatic methods used herein. To the best of our knowledge this is the first work proposing fungal metabolites as BET inhibitors.

Identification of mycorrhizal symbiosis specific autophagy genes under TOR signal disruption in common bean

Elsa-Herminia Quezada[1], Manoj-Kumar Arthikala[1], Miguel Lara[2], Kalpana Nanjareddy[1]*

[1]Ciencias Agrogenómicas, Escuela Nacional de Estudios Superiores, Universidad Nacional Autónoma de México (UNAM), León, C.P. 37684, Guanajuato, Mexico.
[2]Instituto de Biotecnología, Universidad Nacional Autónoma de México, v. Universidad 2001, Chamilpa, 62210 Cuernavaca, Morelos, Mexico. *Corresponding author: kalpana@enes.unam.mx ; Tel. 01(477) 194 08 00 Ext.43462

Autophagy is catabolic pathway to maintain the cellular homeostasis in eukaryotes. During autophagy, the cells form double-membrane-bound vesicle named the autophagosomes and target their own constituents for the turnover of damaged proteins, organelles and toxic components. Unlike in animal systems the knowledge of ATG gene function and their mode of action in plants are not well understood. Studies in Arabidopsis provide some information about autophagy in plants however; the role of autophagy during symbiosis is yet to be deciphered. Recently, we reported the involvement of TOR (target of rapamycin) kinase in rhizobia infection process and nodule development in crop legume, common bean. Interestingly, TOR signaling is known to negatively control autophagy process. Herein, to address the knowledge gaps we performed quantitative transcriptome analysis of mycorrhiza colonized common bean roots and TOR-RNAi roots; and integrated the data to identify potential candidate genes that participate in mycorrhiza-legume interaction. Our results show presence of 30 autophagy-related genes in the common bean genome, which were closely related to the other legume, non-legume and monocot members. Multiple protein alignments indicated the presence of highly conserved domains in these genes. Variable expression patters were observed in different tissues and organs of wild-type common bean. The quantitative expression profiles by RNA-sequencing identify, totally 16 autophagy-related genes that respond to *Rhizophagus irregularis* symbiosis during early stages of symbiosis; of these, 8 genes were upregulated and 8 were downregulated relative to the uninoculated control roots. On the other hand, under TOR silenced (TOR-RNAi) conditions totally 16 ATGs were differentially expressed among them 14 upregulated and 2 downregulated. Based on the integrated approach we have identified 4 potential candidate ATG genes that need to be characterized to understand their precise function during symbiosis. This work is supported by PAPIIT (DGAPA-UNAM) grant no. IA205117 to MK.A, IN219916 to M.L. IN211218 to K.N and CONACYT fellowship grant no. 409344/289810 to EH.Q.

Genetic and physiological interactions between the mitochondrial protein Slm35 and the components of the autophagy machinery

Josué M.J. Ramírez-Reyes & Soledad Funes
Departamento de Genética Molecular, Instituto de Fisiología Celular, UNAM Circuito Exterior s/n, Ciudad Universitaria, Coyoacán Cd.Mx. 04510 (+52-55) 56225679, sfunes@ifc.unam.mx

Autophagy is a catalytic cellular process that targets cytoplasmic components for degradation into the vacuole. In yeast, there are 40 Autophagy Related Genes (known as *ATG*) that encode for proteins involved in different steps of autophagy. The selective degradation of mitochondria by autophagy is called mitophagy and it is modulated by the selective receptors Atg32 and Atg33. Mitophagy is a quality control process that also works during the oxidative stress response.

Slm35 is a mitochondrial protein involved in longevity and stress responses in *Saccharomyces cerevisiae*. The gene *SLM35* shows genetic interactions with some *ATG* genes during lifespan regulation. Mitochondrial flux is increased in a *Δslm35* mutant. However, it is unclear how Slm35 functions within the autophagy and mitophagy machineries.

In this work, we evaluated possible genetic interactions between *SLM35* and *ATGX* genes by generating double *ΔatgxΔslm35* mutants, where *Δatgx* represents deletions of components involved in general, selective, mitophagy, cytoplasm-to-vacuole and pexophagy autophagy. The *Δslm35* strain shows a characteristic resistance to oxidative stress conditions, however in some of the double mutants this phenotype was lost, suggesting that the resistance to stress in the absence of *SLM35* depends on autophagy.

The role of the histone deacetylases in the morphology and virulence of the fungus *Macrophomina phaseolina* (Tassi Goid).

[a]Ramos-García, Víctor Hugo; aVillota-Salazar, Nubia Andrea; [b]Cortes-Espinosa, Diana Verónica and [a]González-Prieto, Juan Manuel.
[a]Centro de Biotecnología Genómica - IPN. Reynosa, Tamaulipas., Mexico.
[b]Centro de Investigación en Biotecnología Aplicada - IPN. Tlaxcala, Tlaxcala., Mexico.
Corresponding author: vramosg1600@alumno.ipn.mx

Abstract

Fungal phytopathogens require different skills to infect the plants and complete its lifecycle. The strategies to infect the host consist mainly of adaptation to stress conditions, penetration of physical barriers and overcoming the plant defense system. Essentials proteins for pathogenesis are responsible of these skills and its production is regulated by epigenetic mechanism through chromatin-remodeling. Furthermore, there is evidence that these mechanisms participate in the gene-regulation involved in the development of the fungal pathogenic process, because their alteration can reduce the phytopathogens infective ability and virulence. *Macrophomina phaseolina* is an important phytopathogen that leads considerable losses of different crops, especially during drought conditions. For the above, its features of morphology and virulence have been widely studied. However, the epigenetic mechanism function in this fungus has not been elucidated. In the present work, we studied the role of some histone deacetylases by means of chemical inhibition. The *M. phaseolina* morphology and virulence were analyzed under the effect of histone deacetylases (HDACs) inhibitors, valproic acid (VPA) and sodium butyrate (SBT). The main effects in *M. phaseolina* strains with the application of HDACs inhibitors (HDACi) VPA and SBT were a reduction in aerial mycelium production, vegetative growth, change in the cells with pigmentation less dark, mycelium slightly translucent, diameter and number of microsclerotia per mm2. Also, the HDACi VPA and SBT affected significantly ($P < 0.05$) the virulence of *M. phaseolina* in BAT-477 bean variety, reducing germination of the seed by 27%. The inoculation of *M. phaseolina* caused death in the bean seedlings before and after the emergence, mainly in the variety Pinto-UI114. Among the susceptible materials with a lower yield to *M. phaseolina*, stood out the variety PintoUI-114 and resistant BAT-477. In the BAT 477 bean variety, the percentage of healthy plants in the treatments with the fungus inhibited were higher than negative control.

Isolation and Expression Levels of SRY-box 9 (SOX9) in Harderian Glands of Syrian Hamster (*Mesocricetus auratus*).

Luis Ramos Tavera, Lizette Mares Gómez, Bertha Alicia Chávez Cano and Felipe de Jesús Vilchis Uribe. *Department of Reproductive Biology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, México City, 14080, México. Tel. 54870900 ext. 2423 vilchisuf@prodigy.net.mx*

SOX9 [SRY-related high-mobility-group box gene 9 (*SOX9*)] is a transcription factor essential for sexual development of testes from bipotential gonads, acting as the master regulator of Sertoli cells and testis cords differentiation during embryogenesis. Ocular gland morphogenesis including the Harderian glands (HGs) involves epithelial specialization and acinar differentiation. The HGs develop from the prospective conjunctival and eyelid epithelium, and SOX9 is required for the development of these intraorbital structures. In turn, FGF/Fgf2r signaling is required for the expression of SOX9. It is involved in the maintenance and function of liver, pancreas and cartilage and likely the HGs, since it is expressed during embryonic ocular surface development. In hamsters, the HGs exhibit a clear sexual dimorphism dependent of androgens. The molecular actions of SOX9 to promote the development and maintenance of the peripheral and sexual organs make of this transcriptional factor, candidate to expand our understanding about its role on HG function. The aim of this study was to isolate the full-length cDNA of Sox9, examine its tissue distribution and expression pattern in HGs of male and female hamsters. The full-length Sox9 cDNA sequence (3649-bp) contains an 81-bp 5´ untraslated region (UTR) and a long 3' UTR of 2044-bp, an ORF of 1524-bp with a canonical polyadenylation signal (AATAAA) at 19 nucleotides upstream of the poly(A) tail (GenBank accession number MH337871). The isolated cDNA encodes for a 507 amino acid protein containing the potential DNA-binding domain known as HMG box. BLAST analysis revealed an identity of 99%, 99% and 97% identity with the Sox9 of *Mus musculus* (NP_035578.3), *Rattus norvegicus* (NP_536328.1) and *Homo sapiens* (NP_000337.1), respectively. Real-time qPCR analysis demonstrated that Sox9 expresses more abundantly in the HGs of males than in females. High expression levels were also observed in testis, cerebellum hypothalamus and pancreas. The dimorphic expression pattern suggests that SOX9 plays an important role in regulating the secretions that lubricate and protect the ocular surface as well as the epithelial specification and acinar differentiation in hamster HGs, which probably occur downstream the FGF and androgen signaling.

Mutation Analysis of Aldo-Keto Reductases (*AKR1C2/4)* and *HSD17B6* in Subjects 46,XY with Nonsyndromic Hypospadias.

Luis Ramos Tavera, Lizette Mares Gómez, Bertha Alicia Chávez Cano and Felipe de Jesús Vilchis Uribe. *Department of Reproductive Biology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, México City, 14080, México. Tel. 54870900 ext. 2423* vilchisuf@prodigy.net.mx

Hypospadias is a congenital anomaly that is often associated to molecular alterations in androgen synthesis or action. During embryogenesis, the formation of the male urethra depends largely on the enzyme-mediated conversion of testosterone (T) into 5α-dihydrotestosterone (DHT). Recent studies in marsupials, have suggested that in fetal testis could be operating two distinct metabolic pathways to synthesize this bioactive androgen; 1) the ~~c~~lassic biosynthetic" route (T→DHT) mediated by *SRD5A2*, and 2) an alternative ~~b~~ackdoor" pathway where DHT is synthesized through 3α-HSD reductive reactions mediated by AKR1C2-4 enzymes without T intermediacy. To ascertain whether gene defects of certain 3α-HSD/AKRs impair urethral DHT formation resulting in hypospadias, here we performed molecular studies in four genes of the ~~b~~ackdoor" pathway in normal male subjects (*n*= 50) and karyotypic males with single hypospadias (*n*= 25). Coding regions of *AKR1C2, AKR1C3, AKR1C4* and *HSD17B6* genes were analyzed by PCR-SSCP and Sanger sequencing. Molecular screening studies revealed distinct genotypic patterns at different exons of *AKR1C2, AKR1C3* and *AKR1C4*, while *HSD17B6* presented wild-type sequences. DNA analyses detected two synonyms variants [rs13945, p.D109= and p.H222=] in *AKR1C2,* all without apparent phenotypic expression. In *AKR1C3* were identified two single nucleotide polymorphisms (SNP), p.H5Q (rs12529) and p.E77G (rs11551177); two unreported heterozygous variants p.P180S (Possibly damaging), in 4/100 and p.R199Q (Polymorphism), in 2/100 and one silent variant [p.K104=], in 13/100. Two SNPs, p.S145C (rs3829125) and p.L311V (rs17134592) were identified in *AKR1C4*. Together, these results indicate that mutations in *AKR1C2, AKR1C3* and *AKR1C4,* are most likely SNPs, and not, deleterious genetic variants, since these changes were detected in both patients and control healthy subjects. Bioinformatics analysis excluded possible pathogenicity in most of these genetic variants. Although the number of cases examined is limited, the data allow suggesting that inactivating mutations in *AKR1C2, AKR1C3, AKR1C4* and *HSD17B6* are an infrequent cause of hypospadias, which would weaken a possible contribution of the ~~b~~ackdoor" pathway for embryonic urethral masculinization.

Transcriptional network evolution underlying biofilm formation in *Candida maltosa*

Aníbal Uriel Reyes Mérida; Eugenio Mancera Ramos.

Departamento de Ingeniería Genética, CINVESTAV - Irapuato. Km. 9.6 Libramiento Norte Carr. Irapuato-León 36824 Irapuato Gto. México Tel: +52 (462) 62396 65, eugenio.mancera@cinvestav.mx

Gene expression is regulated through the direct binding of transcription factors (TFs) to cis-regulatory genomic DNA sequences usually located in the upstream region of genes. Changes in any of the components of such transcription circuits lead to modification in gene expression patterns which underlie many of the phenotypic differences within and between species. It has been observed that modifications in transcription circuits between species are common. However, many of these changes produce equivalent expression patterns. Therefore, the possible adaptive value of such changes has been questioned. To gain insight into the evolutionary processes that shape transcription circuits and more specifically into the role that the environment plays in their evolution, we characterized the transcription circuit that controls the formation of biofilms in *Candida maltosa*. This species of ascomycete yeast is closely related to *Candida albicans* and *Candida tropicalis,* two common opportunistic human pathogens, but has never been isolated from humans. Instead, *C. maltosa* is encountered in industrial environments, a very different ecological niche than the human body. In *C. albicans* and *C. tropicalis,* biofilm formation is thought to be an essential phenotype for the colonization of the human host. Characterization of biofilm formation in *C. maltosa* showed that this species forms biofilms that are much thinner than those formed by its related species. However, *C. maltosa* was able to form filaments which is a distinctive component of *C. albicans* and *C. tropicalis* biofilms. One of the main TFs regulating biofilm formation in *C. albicans* is Efg1. To characterize the role of this TF in *C. maltosa* we developed a system to genetically modify this species and generated gene knockout mutants of Efg1. These mutants were unable to filament and formed lighter biofilms than the WT strain. Thus far, our results suggest that the transcription factor Efg1 has a conserved function in *C. maltose* despite inhabiting a very different ecological niche than its closely related pathogen species. Apart from showing how the biofilm transcription circuit evolved, this work will help us understand the importance of biofilm formation for the ecology of these fungi.

Probing the phosphoryl-group transfer routes in the ArcB dimer

Rodríguez Rangel,C., Terán Melo J.L., Alvarez, A. F.,Georgellis, D.*

Departamento de Genética Molecular, Instituto de FisiologíaCelular, Universidad Autónoma de México, México, D.F., 04510, México.Teléfono: (52) 5556225738. E-mail: crangel@.ifc.unam.mx.

The Arc (Anoxic redox control) two-component system is used by bacteria to modulate the expression of numerous genes in response to respiratory growth conditions. This system, comprises the response regulator ArcA and the sensor histidine kinase ArcB. Under reducing growth conditions, ArcB autophosphorylates and then transfers its phosphoril group to ArcA through a His292→Asp576→His717→Asp54 phosphorelay. Phosphorylated ArcA (ArcA-P), in turn, represses the expression of many operons involved in respiratory metabolism and activates others that encode proteins involved in fermentative metabolism. On the other hand, under aerobic growth conditions, ArcB dephosphorylates ArcA-P through an Asp54 → His717→ Asp576 → Pi reverse phosphorelay.

It has been previously reported that ArcB, in contrast to most homodimeric sensor kinases, autophosphorylates through an intramolecular reaction, requiring both the ATP-binding and the site of autophosphorylation to be present in the same ArcB molecule. However, the mode of phosphoryl-group transfer in the subsequent steps of the phosphorelay remains unclear. Here, we present the results of experiments aiming at elucidating the phosphoryl-group transfer routes with regard to signal transmission and signal decay.

Characterization of the promoter of *MYO1G* and *MYO1F* human: Gene regulation of basal transcription

Rosa Isela Rodríguez Télleza,c, Dr. Guillermo Aquino Jarquínb, Dra. Rosa María Ribas Jaimesa y Dr. Genaro Patiño Lópezc.
aLaboratorio de Producción y Control de Biológicos del Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional. bLaboratorio de Investigación en Genómica, Genética y Bioinformática. Hospital Infantil de México, "Federico Gómez" y cLaboratorio de Investigación en Inmunología y Proteómica. Hospital Infantil de México, "Federico Gómez". Dr. Márquez 162, Col Doctores, C.P. 06720, Ciudad de México, Tel: 52289917 Ext. 4507, correo electrónico: gena23pat@yahoo.com

Class I myosins are a subfamily of motor proteins, composed of 8 members (1a-1h) that participate in various cellular processes related to the cytoskeleton and events related to the cell membranes. Myo1g and Myo1f members of this class, are proteins that are highly expressed in T and B lymphocytes. It is known that Myo1g is exclusively hematopoietic and is expressed in the plasma membrane and in the microvilli of these lymphoid cells, it is also important for the immunological synapse between the T lymphocyte and the dendritic cell. The deficiency of this protein affects the migration and adhesion of the T and B lymphocytes. While Myo1f is also expressed in important ways in neutrophils, and the deletion of the gene affects cell adhesion and makes it more susceptible to bacterial infections in the mouse. However, the deficiency of these two proteins in human cells is not known. Interestingly, gene expression reports show that *MYO1G* and *MYO1F* are significantly over-expressed in acute B-cell lymphoblastic leukemia (B-cell ALL), compared to other types of cancer.
Leukemia is a public health problem worldwide, and in Mexico based on 2012 reports every year the incidence is from 5 to 6 cases per 100 thousand inhabitants. This type of cancer affects cells of the immune system including T and B lymphocytes, with B-cell ALL being the most frequent in children under 18 years of age in countries such as Mexico. This cancer arises from different factors related to exposure to the environment and genetic susceptibility. Various cellular processes are altered in the development of cancer, some related to molecular mechanisms involved in the control of gene expression, such as transcriptional regulation, post-translational modifications, epigenetics among others.
In this work, the promoter of *MYO1G* and *MYO1F* was characterized in human leukemia cells at the level of the regulation of basal transcription, since it is the first important step in the control of gene expression. To do this, genetic engineering tools were used among others, and in this way, more details of the regulation of the gene expression of these two human myosins are provided to our understanding

Search for genes involved in degradation of Benzo[a]pyrene in *Bacillus licheniformis* M2-7

Augusto Rojas-Aparicio, José A. Hernández-Eligio, Jeiry Toribio-Jiménez, Miguel Á. Rodríguez-Barrera, Yanet Romero Ramírez and Carlos Ortuño Pineda*.
Nucleic Acid and Protein Laboratory, Autonomous University of Guerrero, México. Ave Lázaro Cárdenas. Ciudad Universitaria, 39070, Chilpancingo, Mexico.
*Corresponding author: Carlos Ortuño Pineda
E-mail: extra_class007@hotmail.com

Abstract
Bacillus licheniformis M2-7 is a heat-resistant bacterium able to biotransform polycyclic aromatic hydrocarbons. It can transform a wide range of these compounds as naphthalene, phenanthrene, pyrene and benzo[a]pyrene. Benzo[a]pyrene is a polycyclic aromatic hydrocarbon of high molecular weight considered as potentially toxic and carcinogenic for humans. Aiming to discover the genes involved in the biotransformation of benzo[a]pyrene, we made a B. licheniformis M2-7 genomic library in E. coli. We isolated two E. coli strains that were able to grow in minimal salt medium supplemented with benzo[a]pyrene. From the analysis of the DNA fragments in the clones H23 and H38, we identified open reading frames coding for 5 possible genes, among them pobA and fabHB, which products are the enzymes 4- hydroxybenzoate 3-monooxygenase and the ketoacyl-ACP synthase III, respectively. To evaluate the role of these genes in the metabolism of benzo[a]pyrene in B. licheniformis M2-7, we estimated their relative expression through reverse transcription quantitative PCR. Finally, we observed that the genes pobA and fabHB were overexpressed after 3 h under induction with benzo[a]pyrene, suggesting that this strain could use these genes during the metabolism of this PAH, plus it does it in a faster time than that reported for other bacterial genera.

Key words: 4-hydroxybenzoate 3-monooxygenase (*pobA*); ketoacyl-ACP synthase III (*fabHB*); *Bacillus licheniformis* M2-7; benzo[a]pyrene; reverse transcription quantitative PCR.

"Nuclear organization of breast cancer oncogenes: a new approach using CRISPR-dCas9 technology"

Pablo Antonio Rojas Reyes and Lorena Aguilar Arnal*
* Instituto de Investigaciones Biomédicas, Sede Tercer Circuito Escolar, Apartado Postal 70228, Ciudad Universitaria 04510, México, CDMX. Oficina 562-28938.
loreaguilararnal@iibiomedicas.unam.mx

Nowadays, it is generally accepted that gene expression happens in a three-dimensional context, where the transcription is regulated by the interaction of dozens of trans- and cis-acting sequences, the conformational state of chromatin, recruitment of transcription factors, the response to external signals and the functional and energetic needs of the cell. All this knowledge has initiated interesting fields of research in genomics, epigenetics and epigenomics, with results that can be interpreted in health and disease contexts. This area of research can lead to applications in the treatment of diseases such as cancer (1). This project intervenes in these investigations, with the implementation of a study method based on CRISPR (2), which allows to visualize *in vivo* the location of specific loci in the cell nucleus through a novel system and with practical and economic advantages over current methods (FISH or CISH).
Aims of de study. Develop a CRISPR-dCas9 protocol in cancer cell lines to visualize the location of oncogenes with clinical importance and whose amplification has been reported in the development and prognosis of the disease
Method and results. The three-dimensional reconstruction of the nucleus and the *in vivo* labeling of the alleles of the *HER2* gene allows us to have a clearer idea about the way in which *HER2* is organized inside the cell nucleus in breast cancer cell lines with distinct levels of *HER2* amplification.

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Study of light response in *Metarhizium* spp.

Adriana García Tapia, Abraham A. Gasca-Venegas, Gloria Angélica González Hernández, Araceli López Andrade, Israel E. Padilla Guerrero and Juan Carlos Torres-Guzmán.

Universidad de Guanajuato, Campus Guanajuato, División de Ciencias Naturales y Exactas. Colonia Noria Alta s/n, Guanajuato, Gto.; México C.P. 36050. Tel. (473) 7320006 ext. 8160. Correo electrónico: torguz@ugto.mx

Metarhizium spp. are entomopathogenic fungi used as biological pest control of important crop plants like corn, wheat, broccoli, among many others. Some fungi make interactions with plants in the rhizosphere. *Metarhizium* spp is commonly found in soil and form endophytic interactions with various species of plants, promoting their growth (Behie *et al*, 2012, Liao *et al*, 2014).

Metarhizium infection cycle starts with the conidial adhesion to insect cuticle, followed by germination and penetration into host hemocoel, where fungal cells are rapidly propagated by budding until host death, the cycle is terminated by the production of conidia on the cadaver surface under appropriate conditions. Since unicellular conidia produced on solid substrates are the active ingredients of traditional myconsecticides, their responses to stresses, such as high temperature and solar UV irradiation, are of particular concern for the success of a fungal formulation applied in insect control.

In several species of fungi, it has been described how the light is involved in various biological processes: growth, pigment formation, circadian clock, and photoconidiation. In fungi such as *Aspergillus nidulans*, *Trichoderma reesei*, Trichoderma *atroviridae* and *Neurospora crassa* light-response genes have been reported, encoding proteins involved in light perception, promoting the growth and formation of conidia. The study of how light affects the development of the fungus *Metarhizium* sp., is critical, as it currently has not been done any description of this process and will allow us to understand more about the biology of this fungus, particularly during the asexual reproduction.

In this work, we analyzed the effect of the light during conidiation of nine strains of *Metrhizium* spp., when they are exposed to a photoperiod treatment and a total darkness treatment. We also are analyzing the expression pattern and intracellular localization of the product of the *Cie1* gene, which is involved in photoconidiation. *Cie1* contains a particular PAS domain of the photoreceptor proteins.

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Characterization of differentially expressed long non-coding RNAs during the interaction between adipose-derived stem cells and cervical cancer cells.

Victor Hugo Rosales-Gallegos, Rosario Castro-Oropeza, Jorge Melendez-Zajgla, Vilma Maldonado-Lagunas.
Epigenetics Laboratory, Instituto Nacional de Medicina Genómica.
Periférico Sur 4809, Arenal Tepepan, Tlalpan, Ciudad de México, C.P. 14610.
vmaldonado@inmegen.gob.mx +52 55 5350-1900 ext.1140

Introduction. Accumulating evidence has shown that there are cervical cancer associated risk factors supplementary to human papillomavirus infection. Among those additional factors, obesity and overweight stand out since they have been related with a higher incidence and mortality of different types of cancer. Besides it has been reported that cervical cancer patients with high body mass index (BMI) present a twice-increased risk of cervical adenocarcinoma as well as a worse prognosis1.

Multiple research papers have evinced that adipose-derived stem cells (ADSC), contribute to the malignant development because of the recruitment of ADSC into the tumor site. These cells, particularly abundant in obese related adipose tissue stroma in obesity conditions, enrich the tumor microenvironment trough the secretion of cytokines and growth factors that enhance the invasiveness of the cells as well as their metastatic and proliferative capabilities2.

Objective. The aim of this research work is to reveal the long non-coding RNAs (lncRNAs) involved in the interaction between cervical cancer cells and ADSC.

Results. Co-culture assays of ADSC and cervical cancer cell line HeLa were performed. Thereafter the transcriptome of HeLa cells alone or in co-culture with ADSC was analyzed using Illumina platform, finding 483 upregulated and 337 downregulated lncRNAs. Then, the expression of five of the top lncRNAs was validated using droplet digital PCR technology further identification of subcellular fraction abundance of the validated lncRNAs through cell fractionation and RT-qPCR quantification. This was made with the purpose to elucidate the potential functional mechanism of this lncRNAs based on its subcellular localization. Also, the expression levels of these transcripts were assessed in a panel of four cervical cancer cell lines versus an immortalized non-malignant cell line. Moreover, inhibition of the major signaling pathway involved in the ADSC-HeLa interaction was performed, followed by the expression quantification of the validated transcripts. Finally, by means of public available clinical data, we found a correlation between the expression of one of the described lncRNAs with the overall survival of cervical cancer patients.

Conclusion. Characterization of aberrantly expressed lncRNAs in the ADSC – Cervical Cancer cells interaction opens a window in the search of potential cervical cancer biomarkers in the association between overweight/obesity and cervical cancer screening.

Key words: Adipose-derived stem cells; Cervical cancer; LncRNA; ddPCR.

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Functional characterization of mutations identified in the LMNA gene associated with dilated cardiomyopathy in Mexican patients

Sandra Rosas-Madrigal, Arturo Rojo-Dominguez, Sandra Romero-Hidalgo,
Rigoberto Rosendo-Gutiérrez, Enrique Lopéz-Mora, Alessandra Carnevale.
Instituto Nacional de Medicina Genómica (INMEGEN)
Periférico Sur 4809, Col. Arenal Tepepan, Tlalpan.
Ciudad de México 14610
acarnevale@inmegen.gob.mx

Lamin A/C are important proteins involved in structural integrity of the nuclear envelope, chromatin organization within the nucleus and the DNA transcription. Lamins interact between each other and with other proteins of the inner nuclear membrane, including lamina-associated-polypeptide 2α (Lap2α), also named thymopoietin (TMPO).
Multiple mutations in the *LMNA* gene cause hereditary diseases called laminopathies that mainly affect: striated muscle, adipose tissue, peripheral nerve with features of accelerated aging and isolated dilated cardiomyopathy.
Cardiomyopathy caused by *LMNA* mutations has a relatively rapidly progressive course with sudden death from arrhythmias and the onset of heart failure occurring at earlier ages as compared with most of the other inherited cardiomyopathies. Dilated cardiomyopathy (DCM) is a heterogeneous disorder of the cardiac muscle characterized by left or biventricular dilatation with systolic dysfunction in the absence of abnormal loading conditions such as coronary artery disease or hypertension and an ejection fraction <45% (Hariharan R., 2011). The major clinical manifestation is the heart failure often associated with arrhythmia and sudden cardiac death and it is considered the primary indication for cardiac transplantation.
Approximately 30-50% of the affected subjects have a familial form, generally inherited with an autosomal dominant pattern (90%), and less frequently with an autosomal recessive and X-linked transmission.
Next generation sequencing (NGS) approaches have contributed to the tremendous advances in the identification of genetic causes of DCM in developed countries. In Mexico City, we recently initiated a cohort study of patients with sporadic or familial DCM using NGS for the clinical genetic testing. Here, we are here presenting a Mexican family with a severe form of DCM where a mutation in the *LMNA* gene is segregating with a common variant in the *LAP2α* gene. We characterized the molecular and subcellular changes in the protein induced by the mutations in the *LMNA* gene, modifications in the gene and protein expression, in the protein structure by using an *in silico* model and changes in the interaction dynamics of lamin protein with other proteins.

Genetic edition in filamentous fungi mediated by CRISPR: *Sclerotium cepivorum* Berk and *Trichoderma atroviride*

Luis Mauricio Salazar García [1], Sandra E. González Hernández[1], José A. Martínez Álvarez1, Vianey G. Olmedo Monfil[1], Alfredo Herrera Estrella[2], Patricia Ponce Noyola[1]
[1]. Departamento de Biología. Universidad de Guanajuato. Noria Alta S/N. Col. Noria Alta, C.P. 36050. Guanajuato. México. Tel:52(473)732006 Ex 8149. Email: poncep@ugto.mx
[2]. Laboratorio Nacional de Genómica para la Biodiversidad, Centro de Investigación y de Estudios Avanzados del IPN Sede Irapuato, Irapuato 36821, Guanajuato, México. Email: aherrera@langebio.cinvestav.mx

Emerging strategies to study gene function such as the recently developed CRISPR-Cas9, are excellent tools to modify microbial genomes for which it is difficult to obtain stable transformants. The possibility of genetically manipulating microorganisms allows in depth studies of biological processes. In our group we are interested mainly in two plant – associated fungi; one of them pathogenic and the other a beneficial plant endophyte.

White rot of *Allium* plants, a disease caused by *Sclerotium cepivorum* Berk is highly persistent because the fungus produces resistance structures known as sclerotia, which remain viable for up to 20 years in the soil. Sclerotia resistance is mainly attributed to the layer of melanin that covers these structures. Scitalone dehidratase (SDH) is a as key enzyme in melanin production in this fungus, which contains an NHEJ domain important part for its catalytic activity.

Trichoderma atroviride is a plant symbiont that has also been used to study developmental processes such as conidiation in response to light. Light receptors encoded by the *blr-1* and *blr-2* genes are key elements for light-induced conidiation. Blr-1 works through the three PAS domains, one of them with a LOV subdomain, where a conserved cys is a fundamental part of the blue light perception complex.

In order to apply the CRISPR technique in our study models, we directed CRISPR towards the NHEJ domain in the SDH from *S. cepivorum*, this edition will lead to non-pigmented sclerotia production, which could be considered as the phenotypic marker of the CRISPR functionality. Whereas in *T. atroviride,* the edition will be a point mutation, resulting in a cys to gly amino acid change, in the LOV domain of the *blr1* gene. This change will affect its capacity to perceive light, and therefore to produce conidia, representing also a good phenotypic marker of successful genome manipulation.

Up to now, Hyg-resistant transformants have been obtained with a plasmid with Cas9 and the episomal maintenance sequence AMA1 for both organisms. We sinthesized the gRNA for S.c. Berk and *T.a* by fusion PCR from the plasmid pFC334. Primers with the SP6 RNA polypeptide recognition sequence have been designed to allow *in vitro* synthesis of the gRNA, in addition we have cloned a fungal optimized Cas9 in an expression vector and, obtained the Cas9 recombinant protein, all this to carry out the transformation with RNPs.

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“Effects of CBFβinhibition by CRISPR-Cas in a breast cancer cell line”

Ixchel Maritrini Salgado Carranza, Jorge Meléndez Zajgla,
Francisco Javier Gaytán Cervantes y Magali Espinosa Castilla.
Instituto Nacional de Medicina Genómica
Av. Periférico Sur. 4809. Col. Arenal Tepepan. CP. 14610. Ciudad de México.
Tél. 5553501967 email: mespinosa@inmegen.gob.mx

Core binding factor (CBF) is a DNA-binding transcription factor of genes related to development, mainly in embryogenesis, hematopoiesis, and bone development. CBF is composed of a DNA binding RUNX subunit (RUNX1-3) and a non-DNA binding CBFβ subunit, which modulates RUNX protein activity by modulating the auto-inhibition of the RUNX subunits.

Furthermore, as with many genes critical for development, the CBF genes are also involved in carcinogenesis. Alterations in CBFβ expression and significant mutations have been reported in different types of cancer, such as liver, cervical and breast cancer. Recently, in whole exome and genome sequence analysis with 100 breast cancer samples, including mexicans samples, we found CBFβ significant mutations (1).

These findings suggest, that there might be a relation between CBFβ and tumor development. Nevertheless, the biological roles of CBFβin carcinogenesis have not been fully elucidated.

Therefore, to test whether CBFβ is required for the malignant phenotype of breast cancer, we used CRISPR-Cas9 gene editing system to knock out the *CBF* gene in MCF-7 breast cancer cell line. The reduction in CBFβ expression resulted in increased proliferation, enhanced multicellular spheroid size and a slight increased in clonogenic capacity.

This report provides evidence about CBFβ roles in breast cancer cells. Further investigations with *in vivo* models would highlight CBF functions.

HOTAIR knockdown inhibits Wnt pathway by re-expression of its negative regulators

Eric Genaro Salmerón Bárcenas, Dra. Berenice Illades Aguiar, Dr. Daniel Hernández Sotelo. Laboratorio de Epigenética del Cáncer, Universidad Autónoma de Guerrero, Chilpancingo Guerrero, México. C. P. 39071,dhernandez@uagro.mx.

Abstract: Wnt pathway promotes cell migration, invasion and inhibition of apoptosis, and it is over-activated in cancer. HOTAIR is a long non-coding RNA that promotes carcinogenesis through several mechanisms. Recently, it was reported that HOTAIR promotes activation of Wnt pathway in cancer. However, little is known about molecular mechanisms involved. Objective: To determine the role of HOTAIR in Wnt pathway. Methods: HOTAIR knockdown was performed using a DsiHOTAIR in HeLa cells. Wnt pathway was analyzed through luciferase assay, RT-qPCR and western blot. Expression and methylation levels were determined by western blot, RT-qPCR and MSP. 5-hydroxymethylcytosine (5hmC) level was determined by glucosylation followed by restriction and qPCR. Results and discussion: HOTAIR knockdown decreased WNT pathway activity. Moreover HOTAIR knockdown decreased methylation level and increased expression of negative regulators of WNT pathway: PCDH10, SOX17, AJAP1 and MAGI2. These results suggest that HOTAIR is involved in regulation of expression and methylation of this genes. Therefore, we analyzed the role of HOTAIR in DNMTs and TETs expression, enzymes involved in methylation and de-methylation. We found that HOTAIR knockdown decreased DNMT1 expression and increased TET1 and DNMT3A expression. Finally, we determined 5hmC level in PCDH10, SOX17, AJAP1 and MAGI2 promoters. We found that HOTAIR knockdown increased 5hmC level in SOX17 and MAGI2 promoters. In this context, it has been reported in colon cancer that TET1 re-expression increases 5hmC level in DKKs and SFRP2 promoters (negative regulators of WNT pathway). Conclusion: These results suggest that HOTAIR modulates the Wnt pathway by affecting the methylation and expression of its negative regulators

Analysis of miRNAs and their relationship with molecular pathways of signaling to apoptosis, autophagy and inflammation, during the process sarcopenic in quadriceps femoral muscle tissue.

M. en C. Jorge Sánchez Cedillo[1], M.C Aramara Yasmin Aco Flores[1], Dr. José de Jesús Rivera Sánchez, Dr. Miguel A. Fonseca Sánchez[1], Dra. Gloria E. Queipo Garcia[1*].
[1]Hospital General de México —DrEduardo Liceaga", México City, México. Dr. Balmis 148, Doctores, 06720 Ciudad de México, CDMX.
Corresponding autor email: gqueipo99@yahoo.com

Introduction. - Aging is a biological process of all organisms, this entails a series of structural and functional changes that develop progressively with the passage of time. One of the problems that develops during aging is the loss of skeletal muscle mass, this phenomenon is also known as sarcopenia, sarcopenia is a multifactorial process, this loss of muscle mass has as a consequence the loss of strength and muscle function, increasing the rate of fractures and falls putting the life of the elderly at risk, currently a problem is that diagnostic tools are only functional when sarcopenia is already is present, so the study at the molecular level of sarcopenia is important and in the future develop new tools for early diagnosis or new therapeutic strategies. Our working group has observed that there are changes at the level of RNA in the expression of genes related to apoptosis, autophagy and inflammation among frail and functional older adults, one mechanism that could explain these changes is the effect of the elements of regulation of gene expression, such as microRNAs. MicroRNAs (miRNAs) are short 20–22 nucleotide RNA molecules that are negative regulators of gene expression in a variety of eukaryotic organisms, It has been seen that miRNAs are actively involved in muscle development and can vary in conditions such as exercise or disease.
Aim. - Determine the microRNAs that regulate the expression of genes of apoptosis, autophagy and inflammation during the sarcopenic process in muscle tissue of the quadriceps femoris
Results. - From femoral quadriceps muscle biopsies, the differential analysis of the miRNAs of fragile and functional adult sample was performed, where 35 miRNAs were obtained, which are potential elements as markers of sarcopenia. The miRNAS have a higher fold change to 2 and a p-value less than 0.05. The target genes of the miRNAs were searched in the TargetScan platform and a database of all the genes modulated by the selected miRNAS was generated, obtaining 16,002 genes. To help us to understand the way in which differentially expressed miRNAs are interacting in sarcopenia, we selected 8 miRNAs and we analyzed the target genes of the miRNAs and the molecular pathways in which they belong, highlighting the participation of miRNAs in pathways such as MAPK, TGF beta and FOXO. These molecular pathways are related in signaling processes such as apoptosis, autophagy and inflammation.
Conclusion. – We found miRNAS with differential expression among fragile and functional older adults, these miRNAs have been related to genes of apoptosis, autophagy, inflammation and muscle.

Phosphate deficiency negatively affects early steps of the symbiosis between common bean and rhizobia

María del Socorro Sánchez-Correa [1], María del Rocío Reyero-Saavedra [1], Mariel C. Isidra-Arellano [1, 2], Lise Pingault [3], Sidharth Sen [4, 5], Trupti Joshi [4, 5, 6], Norma A. Castro-Guerrero [7], David G. Mendoza-Cózatl [7], Marc Libault [3], Oswaldo Valdés-López [1*]

[1] Laboratorio de Genómica Funcional de Leguminosas. Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México. Tlalnepantla, Estado de México, 54090, México. 2 Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México. Coyoacan, Ciudad de México, 04510, México. [3] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Beadle Center, Lincoln, 6858803, USA. [4] Informatics Institute, C. S. Bond Life Sciences Center, University of Missouri, Columbia, MO, 65211, USA. [5] Christopher S. Bond Life Sciences Center, University of Missouri, Columbia, MO, 65211, USA. [6] Department of Health Management and Informatics, School of Medicine, University of Missouri, Columbia, MO. 65211, USA. [7] Plant Sciences, Christopher S. Bond Life Sciences Center, University of Missouri, Columbia, MO, 65211, USA.

* Av. De Los Barrios 1, Los Reyes Iztacala Tlalnepantla Estado de México, México CP 54090. Phone: 56231333 Ext. 39836. Email: oswaldo_valdesl@yahoo.com.mx

Abstract

Phosphate (Pi) deficiency reduces nodule formation and development in different legume species including common bean. Despite the significant progress in the understanding of the genetic and metabolic responses underlying the adaptation of nodules to Pi deficiency, it is still unclear whether this nutritional deficiency interferes with the molecular dialog between common bean and rhizobia, if so, what part of the molecular dialog is impaired? Here, in this study, we provide evidence demonstrating that Pi deficiency negatively affects critical early molecular and physiological responses required for a successful symbiosis between common bean and rhizobia. For instance, we demonstrated that the expression of PvNSP2, PvNIN, and PvFLOT2, was significantly reduced in Pideficient common bean seedlings. In addition, we showed that the rhizobiainduced root hair deformation was significantly reduced in Pi-deficient seedlings.

Further transcriptional analysis by messenger RNA-sequencing revealed that the expression of hormones and signal transduction-related genes is compromised in Pi-deficient seedlings inoculated with rhizobia. Additionally, we showed that regardless of the presence or absence of rhizobia, the expression of PvRIC1 and PvRIC2, two genes participating in the autoregulation of nodule number, was higher in Pi-deficient seedlings than in control seedlings. The data presented in this study shed light on the understanding of how Pi deficiency impacts in the early steps of the symbiosis between common bean and rhizobia.

LINC00052 roles in MCF-7 breast cancer cells, a migration inhibitor.

José Manuel Sánchez, Edna Mandujano, Alfredo García, Laura Lozada, Cecilia Zampedri, Jorge Meléndez, Vilma Maldonado, Floria Lizárraga. Instituto Nacional de Medicina Genómica, Av. Periférico Sur. 4809. Col. Arenal Tepepan. CP. 14610. Ciudad de México. Tel. 53501188. fjlizarraga@inmegen.gob.mx

Background/aims: Recent studies have shown that long-non-coding RNAs (lncRNAs, a class of transcripts with lengths>200nt) play key roles in tumor progression. Previous work in our lab described a whole transcriptome analysis during multicellular spheroid (MCS) formation. Interestingly, the expression of a specific lncRNA, LINC00052, was strongly inhibited[1]. Moreover, previous data reported that LINC00052 expression is diminished in triple-negative human breast cancer samples, while it is expressed in luminal subtypes[2]. However, LINC00052 functions in breast cancer cells are poorly understood. In this study, we aimed to analyze LINC00052 roles on MCF-7 breast cancer cells.

Methods/Results: LINC00052 expression was decreased in MCF-7 MCS (2-6 days) in comparation to monolayers as analyzed by RT-qPCR, supporting our previous results[1]. Loss of function studies were performed to evaluate LINC00052 relevance in MCF-7 cellular processes. Microarray expression assay (Affimetrix chip HuGene-2_0-st-v1) was done in order to elucidate genes and cellular functions modified upon LINC00052 knockdown, followed by RT-qPCR to verify differential expression. Data was analyzed with the Transcriptome Analysis Console Affimetrix tool. A total of 933 genes were found to be differentially expressed, 638 were overexpressed and 295 were diminished (Fold change>2, P<0.05). Ingenuity Pathway Analysis tool inferred that 6 canonical cellular pathways related to cell cycle control and DNA repair machinery were modified. In addition, cellular signaling networks related to cell movement were identified. Furthermore, Gene set enrichment analysis (KEGG, GO y REACTOME) showed that mitochondrial related processes (oxidative phosphorylation and electron transport chain), cell cycle and DNA repair systems were enriched.

Next, a series of *in vitro* assays were used to observe LINC00052 functions in MCF-7 cells. First, we measured oxygen consumption rates upon LINC00052 knockdown, however, no significant difference with control shRNA was observed. Next, *in vitro* cell migration capacity was evaluated employing transwell assay. We observed that MCF-7 cells had higher migration ability when LINC00052 expression was inhibited. In addition, MCF-7 cells showed a greater gelatin degradation capacity upon LINC00052 knockdown. Finally, using a xenograft migration model in zebrafish we observed a higher migration capacity *in vivo*, when LINC00052 expression was inhibited.

Conclusions: Our work shows that LINC00052 might regulate some cellular processes that allow tumor progression and adds light about lncRNA functions in BC progression.

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MIR-7 AND ITS TARGET GENES EXPRESSION IN BREAST CANCER CELL LINES.

David Sánchez Marín, Eduardo López Urrutia, and Carlos Pérez Plasencia*
Laboratorio de Genómica Funcional, Unidad de Biomedicina, Facultad de Estudios Superiores (FES) Iztacala, Universidad Nacional Autónoma de México (UNAM), Avenida de los Barrios Número 1, Colonia Los Reyes Iztacala Tlalnepantla, Estado de México, C.P. 54090.
* carlos.pplas@gmail.com

Breast cancer is the second type of cancer that causes deaths in Mexico and worldwide. One of the hallmarks of cancer is metabolism reprogramming which provides the cancer cell with more nutrients to satisfy de high energy demand interfering many signaling pathways and key enzymes. Some of these enzymes are ACADL, CSRNP3, PCK1 and PDK4, involve in β-oxidation, metabolic syndrome, glycogenesis, and glucose metabolism respectively. These enzymes are target of different regulatory elements such as MicroRNAs. The microRNAs that downregulate tumor suppressor genes are called oncomirs, and the microRNAs that downregulate oncogenes are called tumor suppressor microRNAs. MiR-7 has been reported as tumor suppressor gene in several types of cancer such breast, gastric and brain participating in angiogenesis, proliferation and metastasis. MiR-7 also develops an oncogene role in breast cancer promoting invasiveness and tumor agresivity. Through a bioinformatic research, our work group found that MiR-7 was under regulated in cancer and that the enzymes mentioned before were likely its target genes. Due to the metabolic function of these genes, it is of our interest to verify the interaction between miR-7 and its putative targets, and its possible influence on the metabolism of breast cancer. The objective of this work was to determine the expression of miR-7 and the four aforementioned target genes in different breast cancer cell lines trough quantitative PCR. The analysis suggested a possible correlation between miR-7 and the ACADL, CSRNP3, PCK1 and PDK4 genes.

In silico identification of the putative gene of the RNA subunit of telomerase and analysis of its disrupted mutants in *Ustilago maydis*

Juan Antonio Sanpedro Luna[1], Estela Anastacio Marcelino[2], Candelario Vázquez Cruz2, Patricia Sánchez Alonso[2]
[1]Posgrado en Microbiología, [2]Centro de Investigaciones en Ciencias Microbiológicas. [1,2]Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla. Edificio IC11, C. U., CP. 72570, Puebla Pue. México. Tel: +52 222 229 55 00 ext. 2534 e-mail: maria.sanchez@correo.buap.mx

Introduction: Telomerase is a ribonucleoprotein reverse transcriptase that synthesizes telomeric DNA de *novo* and solves the so-called problem of the terminal replication of linear chromosomes. Its central components are the TERT (telomerase reverse transcriptase) protein subunit that is the catalytic subunit, and TER (telomerase RNA), the RNA subunit that carries the template for the retro-transcription of the telomeric repeats and serves as scaffold for telomerase assembly[1]. Unlike TERT, which has widely conserved motifs and has previously been identified and characterized in *U. maydis*[2], TER is wide divergent among the different taxa, alike other lncRNAs. TER efficacy is mainly determined by the formation of secondary structures conserved among protozoa, fungi, and metazoan[3]. In this work we focused our efforts to identify the TER gene in *U. maydis*, and to characterize its disruptive mutants. To accomplish these objectives, we used the genome sequences of laboratory strain 521 of *U. maydis* and from 20 basidiomycete fungi, to procced accordingly to Chakrabarti *et al.*[4] for *in silico* analysis of non-coding sequences. For the one-step gene disruption of the putative gene encoding the RNA subunit of telomerase of *U. maydis* (here named *ter114*) we proceed as Fotheringham *et al.*[5] for protoplast transformation. Analysis of non-coding sequences containing CCCUAA motif, and that preserves a strong synteny among basidiomycetes and Ustilaginales, rendered us the putative gene *ter114*, which shows a low percentage of identity among the analyzed sequences, but conserve the TER motifs and domains in the same order. *ter114* putative gene is able to form *in silico* the secondary structures reported for TER of other taxonomic groups, and which are needed for the activity of telomerase. *ter114*-disrpupted strains showed small and slow-growing colonies, besides the accumulation of elongated cells with irregular edges. Mutants show a reduced proliferative capacity that is congruent with the phenotype reported for TER- mutants in other organisms. Our preliminary analysis of data suggests that the locus *ter114* could encode for the RNA subunit of telomerase in *U. maydis* as well as in other Ustilaginales; *ter114* is the most viable candidate of the RNA subunit gene of telomerase in these fungi, however, it will be necessary to continue our studies.

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Fabaceae miR2199 regulates a bHLH transcription factor mRNA in response to water deficit

Carlos Alfonso Sierra-Sarabia1, Sofía Paulette Romero-Pérez1, Oswaldo Valdés-López2, Alejandra A. Covarrubias1, and José Luis Reyes-Taboada1
1Departamento de Biología Molecular de Plantas, Instituto de Biotecnología, C.P. 62210 Cuernavaca, Morelos, México. 2Facultad de Estudios Superior Iztacala. (UNAM).
Corresponding author E-mail jlreyes@ibt.unam.mx tel:(777)3291668

Plant miRNAs are small RNA molecules (21-24 nt in length) that mediate different processes such as development, stress responses and self-regulatory pathways at the spost-transcriptional level. In a previous report from our group, miRNA2199 (miR2199) was reported as a conserved *Fabaceae* family miRNA that increases its accumulation in response to drought, NaCl, and ABA treatments. *In silico* predictions showed three predicted target transcripts annotated as members of the bHLH (basic helix-loop-helix) transcription factor family in *Phaseolus vulgaris.* A first approach to understand the biological relevance of this regulation module was to measure the three target transcripts and miR2199 levels in response to drought treatments. Results showed unrelated patterns between the three transcripts, it seems possible that this regulation module involves another factors or specific conditions that remains elusive. *Medicago truncatula* is a legume that has emerged as an attractive plant model to study the legumemicroorganisms interactions, in part due to the resources and tools available to modify the expression of genes of interest. In this model, Romero-Pérez (2015) reported the presence of four target transcripts with recognition sites for the *M. truncatula* miR2199; however only two transcripts have been experimentally validated as targets. Only one of these targets, here named as bHLH780, has showed an interesting expression pattern that correlates with miR2199 expression in drought treatments. The aim of this work is to define the participation of miR2199 and bHLH780 and identify which pathways and genes are affected by this module and how they are relevant for the *M. truncatula* stress response.

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Transcriptional coupling of base excision repair in sporulating *Bacillus subtilis* cells

Valeria P. Suárez Castro, Mario Pedraza Reyes
Departamento de Biología, Universidad de Guanajuato, Noria Alta, Guanajuato, Guanajuato, CP 36050, México | pedrama@ugto.mx | Tel. (473) 732 0006 (Ext. 8161)

In all the cell types, the integrity of the genetic material it is constantly challenged by a diversity of endogenous and exogenous factors; including, reactive oxygen species (ROS) and ultraviolet radiations (UV). To counteract the cytotoxic and genotoxic effects of these factors evolution has equipped organisms from three domains of life with a multiplicity of DNA repair pathways[1].
When conditions are inappropriate for growth, the Gram-positive microorganism *Bacillus subtilis* activates a developmental pathway that culminates in the production of spores[2]. These differentiated cells are highly resistant to several physical and chemical factors that easily damage the nucleic acids of its vegetative form of life[3]. Although the role of DNA repair has been extensively studied in growing *B. subtilis* cells, relatively less is currently known regarding the manner in which sporulating cells protect its genetic material to assess an efficient sporulation process. A recent report showed that DNA distorting lesions in sporulating *B. subtilis* cells, elicits an Mfd-dependent mechanism that couples transcription with the Nucleotide Excision Repair (NER) pathway[4]. Interestingly, in the absence of external DNA damaging factors, the sole absence of Mfd affected the sporulation process[4]. These results support the notion that processing of spontaneous DNA lesions, including base oxidation and deamination requires Mfd for efficient spore morphogenesis. In this work, experimental evidences derived from genetic and physiological approaches revealed that the interaction of BER-dependent pathways with Mfd is necessary to eliminate spontaneous genetic lesions with a direct impact in *i*) spore morphogenesis, *ii*) avoidance of spontaneous and induced mutations, and *iii*) resistance to oxidative stress.

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Pharmacogenomic algorithm for acenocumarol dosing.

Tomás Eduardo Texis Valencia, María del Carmen Marin Albino. Beatriz Villegas Torres, Cossío-Aranda JE, Xavier Soberon, Vanessa González Covarrubias.
Instituto Nacional de Medicina Genómica. Periférico Sur No. 4809, Col. Arenal Tepepan, Tlalpan, CDMX. C.P. 14610. Tel. (55) 5350-1900 ext. 1212.
vgonzalez@inmegen.gob.mx

Coumarins are prescribed worldwide in about 1% of the population (Pirohamed, M. 2005). In Mexico, 500,000 patients are diagnosed with thrombosis (Secretaria de Salud, 2010) and require acenocumarol (AC), a coumarin with narrow therapeutic index and high interindividual variability. Thus, the pharmacogenetic consortium, PharmGKB, recommends genetic testing for variants, VKORC1*2, CYP2C9*2, and CYP2C9*3 to improve efficacy and decrease toxicity.
Recent studies indicate these genes present wide genetic differentiation among continental populations including Mexican Natives and Mestizos (Peña, V., et al. 2014). Therefore, implementation of current pharmacogenetic practices may not reflect our population needs. In this study we aimed to consider additional gene variants CYP4F2*2 and NQO1*2; to develop a dosing algorithm that complement current pharmacogenetic guidances.
DNA was available (N=90) from patients recruited at the National Institute of Cardiology in 2006 with an INR=2-3 and who were on stable doses of AC. The microarray DMET-Affymetrix was used to genotype VKORC1*2, CYP2C9*2, CYP2C9*3, and CYP4F2*2. In addition, variant NQO1*2 was assessed by RFLP with in-house designed primers.
Statistical analyses were performed using R and Python. We determined allele frequencies and compared them to other populations. Multiple linear regression (MLR) was used to estimate dose (ln) as the independent variable and age, gender, height, weight, BMI, VKORC1*2, CYP2C9*2, CYP2C9*3 and CYP4F2*2 were considered as covariates. We identified variables VKORC1*2, CYP4F2*2 and NQO1*2 as significant to dose prediction and generated the following algorithm.

$$\begin{aligned} ln\ dosis = {} & 1.5 - 0.35\,VKORC1*2 - 0.11CYP2C9*2 - 0.26CYP2C9*3 \\ & + 0.051CYP4F2*2 - 0.048NQO1*2 + 0.15Gender - 0.005Age \\ & + 1.19Height + 0.01Weight - 0.0121\,IMC \end{aligned}$$

The model was validated by ANOVA and to evaluate its accuracy, we compared dose estimated with a Spanish and a Lebanese algorithm previously reported, evaluating the covariation between the predicted dose and the actual dose. Our model showed an accurate dose estimation (R=0.62, p=5.2x10-11) and compared poorly with the Lebanese (R=0.32, p=0.0012) and the Spanish (R=0.34, p=0.0011).
Our study shows, that the inclusion of additional pharmacogenetic variants may improve dose estimation. Also, it highlights the importance of replicating previous investigations in specific populations as both, allele frequency and size effect of a variant determines the relevance of a marker in a population.

Relationship between *MCP-1* A-2518G polymorphism and microvascular complications in Mexican patients with type 2 diabetes mellitus

Amairany Torres Alvarez[1], Eduardo Carrillo Tapia[1], Elisa Irene Azuara Liceaga[1],Máximo B. Martínez[2], Fernando Fernández Perez[2], Areli Y. Perez Navarrete[1], Maria Elizbeth Álvarez Sánchez[1], Lilia López-Cánovas[1]*
[1]Posgraduate Program in Genomic Sciences, Autonomous University of Mexico City.
[2]General Hospital Dr. Juan Ramon de la Fuente. Iztapalapa. Mexico City
*Campus del Valle. San Lorenzo 290, Col. Del Valle, Mexico City, POBox 03100, tel. 1107 0280 ext. 15314, lilia.lopez.canovas@uacm.edu.mx

Diabetes is a major health problem worldwide, affecting the 9.6% of the Mexican population. Around 60% of diabetic patients suffer any type of vascular complication of the disease, which decrease their life quality, increase the disease budget and usually is the direct cause of death for them. Several genes have been identified and characterized as possible biomarkers for diabetes-related vascular complications in Asiatic populations. However, in Mexican population, this is a field of study yet to explore. The polymorphism A-2518G of *monocyte chemoattractant protein-1* (*MCP-1)* gene and its functional effect has been related to diabetes vascular complications. This polymorphism has been characterized in Mexican population for infectious, autoimmune, oncologic and heart diseases, besides insulin resistance, but not in the context of diabetes and vascular complications. In this study, the *MCP-1* A-2518G polymorphism was genotyped, the blood levels of MCP-1 were measured and the relation between these variables and the presence of microvascular complications was assessed in a sample of Mexican individuals with type 2 diabetes mellitus (T2DM) with and without microvascular complications. The participants were recruited in the Iztapalapa General Hospital. The allele frequency of this polymorphism in the diabetic patients without microvascular complications was the same as in the healthy controls, while the ones with microvascular complications had a higher proportion of homozygous for the G allele. These data are similar to those reported in Asiatic populations. The relation between the G allele, the MCP serum levels and the occurrence of vascular complication in T2DM is discussed.

Exploring the epigenetic convergence between tumorigenesis in mammals and seed formation in flowering plants.

Mijael Torres[1], Gloria León--Martínez[1], Amada Salazar[2], Javier Mendiola[1], Cédric Blanpain[3], Jorge Meléndez[2], and Jean--Philippe Vielle--Calzada[*1].
[1]Grupo de Desarrollo Reproductivo y Apomixis, Unidad de Genómica Avanzada Laboratorio Nacional de Genómica para la Biodiversidad, CINVESTAV Irapuato, Irapuato, México. CP 36824;; [2]Laboratorio de Genómica Funcional del Cáncer, Instituto Nacional de Medicina Genómica, INMEGEN, Ciudad de México, México. CP 14610.3Faculté de Médecine, Université Libre de Bruxelles, Belgique.
*vielle@cinvestav.mx

A likely biological similarity between embryos and tumors was experimentally established in 1964 by Leroy Stevens who showed that pluripotent stem cells of blastocysts could sometimes become teratocarcinomas. That same year, Barry Pierce demonstrated that single teratocarcinoma cells were capable of forming a differentiated embryonic body.

Assuming that the main regulatory pathways of tumorigenic development could be related to gene pathways of reproductive origin, malignant tumors might sometimes behave as parthenogenetic somatic embryos. To test this hypothesis, we are exploring the functional convergence between epigenetic mechanisms regulating meiosis, parthenogenesis and genomic imprinting in mammals and flowering plants. We have conducted large--scale comparisons of microarray and RNA--Seq experiments that include transcriptional profiles of human cancer stem cells (CTCs), early breast, colon and pancreas tumor cells (BrTCs CoTCs and PaTCs, respectively), and *Arabidopsis thaliana* wild--type and mutant ovules showing phenotypes reminiscent of apomixis. We have identified a selected collection of 26 imprinted genes and non--coding transcripts that are consistently over--expressed in CTCs and either BrTcs, CoCTs or PaTCs, including transcription factors, solute carriers, and long non--coding RNAs. Some of these are currently being tested in search for new target candidates for cancer gene therapy, using in vitro model systems.

Analysis of the photoreversible fluorescent protein iLOV as a new reporter gene to evaluate promoters in the protozoan *Giardia lamblia.*

María Fernanda Torres-Rojas, Laura Eloísa Morales-Luna, Erick Alcaraz-Carmona Edson Jiovany Ramírez Nava, Víctor Martínez Rosas, Yadira Yazmín Cortés-Morales, Fabiola Fernández Rosario, Saúl Gómez-Manzo y Jaime Marcial-Quino. Instituto Nacional de Pediatría. Laboratorio de Bioquímica Genética, Secretaría de Salud. C.P. 04300. (+52) 55 1084-0900 (ext.1442). fernandtr.unam@gmail.com

Giardia lamblia, is a protozoan that causes the disease giardiasis, which affects humans and a large number of vertebrates. For this reason it has become a parasite of medical importance, because infants are the most susceptible to this disease.
Since *G. lamblia* is an early divergent microorganism, it has been taken as a good model to study gene regulation, because it shares mechanisms with both prokaryotic and eukaryotic organisms. In order to understand the regulation through the analysis from *G. lamblia* promoters, our objective in this work was to analyze a new reporter gene in this parasite; the photoreversible fluorescent protein iLOV, that unlike of the enhanced green fluorescent protein (eGFP), it is not limited to aerobic conditions for its expression(1).
Then, a vector which contains the iLOV gene regulated by the constitutive *α2-tub* promoter [(2)] was constructed. $1x10^6$ Giardia trophozoites were transfected by electroporation and the recombinant trophozoites were selected with 100 µm of puromicin. Trophozoites were grown at 37ºC in TYI-S33 culture médium[(3)] and the expression of iLOV gene was evaluated through confocal microscopy, fluorescence spectrometry and mRNA transcripts (RT-qPCR). The microscopy results showed that the recombinant trophozoites show green color indicating the expression of the iLOV gene, and the fluorescence obtained was 1.9–fold with respect to the control, using *α2-tub* promoter. These first results suggest, that iLOV gene can be used for analysis from Giardia promoters, and that their expression levels can be determined qualitatively and/or quantitatively.

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Evaluación de la actividad transcripcional de la vía de señalización WNT-β-catenina en líneas celulares de cáncer colorrectal

Samuel Trujano Camacho, Jossimar Coronel Hernández, Alma Delia Campos Parra Veronica García Castillo, Eduardo López Urrutia, Carlos Pérez Plasencia.
Universidad Nacional Autónoma de México. Facultad de estudios superiores Iztacala. Avenida de los Barrios No.1. Colonia Los Reyes Iztacala. México, 54090, Mpio. Tlalnepantla, Estado de México.
Floresta 5 Bis Colonia Santiago Ahuizotla samuel.trujano1@gmail.com
77036499

Colorectal cancer (CRC) is the result of the accumulation of genetic and epigenetic changes that transform the glandular epithelial cells of the colon and rectum, in an invasive adenocarcinoma.

The WNT-β-catenin pathway regulates tissue homeostasis of the stem cells in colon cell compartments (colon crypts); therefore, mutations in genes such as APC, PYGO2 or CTNNB1 (β-catenin), lead to initiation of carcinogenesis.

In the present work, we evaluated the activity of the WNT-β-catenin pathway by luciferase assay with the TOP / FLASH vector. We also evaluated the messenger RNA (mRNA) expression of target genes (MMP7, BAMBI, c-myc, VEGFA, EphB4 and LEF-1), and the transcriptional activators β-catenin and PYGO2, by qPCR in CCR-derived cell lines and by ANOVA <0.001 in TCGA (The Cancer Genome Atlas) data.

As results, we observed that the WNT-β-catenin pathway was over-activated in HCT116 and SW620 cell lines, with significant differences (ANOVA P <0.001). However, we did not detect differences in the mRNA expression of transcriptional activators in either TCGA or cell linesexcept for EphB4, which showed a tendency to increase. Moreover, we used a WNT-β-catenin pathway inhibitor and then we evaluated target genes and transcriptional activators expression and did not detect significant differences (t Student P <0.05). We concluded that the expression of these genes is not only dependent on the β-catenin/TCF interaction.

Key words. Colorectal cancer, WNT-β-catenin pathway, β-catenin and PYGO2

Circulating microRNAs profile in plasma patients with spinocerebellar ataxia type 7 (SCA7)

Claudia Valdez-Vargas, Veronica Borgonio-Cuadra, Sandra Romero-Córdoba, Oscar Hernández-Hernández, Alfredo Hidalgo-Miranda, Bulmaro Cisneros and Jonathan J. Magaña.
Laboratory of Genomic Medicine, Department of Genetics, Instituto Nacional de Rehabilitación, Calz. México-Xochimilco No. 289, Col. Arenal Guadalupe, 14389 Ciudad de México (CDMX).
Phone: (+52) (55) 5999-1000, ext.14708; Fax: (+52) (55)5603-9127; E-mail addresses: maganasm@hotmail.com

Background and aim: Spinocerebellar ataxia type 7 (SCA7), a neurodegenerative disease characterized by cerebellar ataxia and retinal degeneration, is caused by a CAG repeat expansion in the *ATXN7* gene coding region. Disease onset and progression is highly variable between patients, thus identification of specific/sensitive biomarkers that can aid in early diagnosis and improve the monitoring of disease progression is an immediate need. Because altered expression of circulating microRNAs (miRNAs) has been shown in various neurological diseases, they could be useful biomarkers for SCA7. In this study, we identify the presence of altered miRNAs in patient's plasma associated with pathogenic phenotypes in SCA7.

Material and Methods: We used high-throughput qPCR analysis (TLDA) to generate miRNAs expression profile in 35 plasma samples of patients with SCA7 and 17 healthy controls. To independently validate the differentially expressed miRNAs we used TaqMan miRNAassay by real-time PCR.

Results: We found 71 upregulated miRNAS in SCA7 patients compared with healthy controls. Interestingly, early onset (EO) patients, the most severe form of the disease, showed an increased alteration in miRNA levels than adult onset (AO) patients when were compared with healthy control subjects separately. We demonstrated that five validated miRNAs (hsa-let-7a, hsa-let7e, hsa-miR-18a, hsa-miR-30b and hsa-miR-132) possesses the diagnostic value to discriminate between healthy controls and patients, using receiver operating characteristic (ROC) analyses.

Conclusion: Circulating miRNAs might provide accessible biomarkers for disease stage and progression and help to identify novel cellular processes involved in SCA7.

RNA-seq approach to study seed dormancy and germination in *Cedrela odorata* L.

Miguel Ángel Vallejo Reyna*, Florencia García Campusano, Claudia Méndez Espinoza, Jocelyn Pérez García, Elena Hernández Flores
Centro Nacional de Investigación Disciplinaria en Conservación y Mejoramiento de Ecosistemas Forestales, INIFAP.
Avenida Progreso, núm. 5.
Col. Barrio de Santa Catarina, C.P. 04010, Coyoacán, Ciudad de México.
*correo electrónico: vallejo.miguel@inifap.gob.mx; teléfono; 01 800 088 2222 ext. 80634

Cedrela odorata L. (Spanish cedar) is one of the most commercially important timber species for the forest industry in the tropical regions of the New World. Because of the physical properties of its wood, there is a high demand for its seed to supply commercial plantations. It is included in the IUCN red list and is under special protection by Mexican environmental laws. Hence, long-term germplasm conservation strategies are necessary in order to guarantee access to this genetic resource in the future. However, Spanish cedar seeds are not susceptible for long-term storage due to rapid loss of vigor and viability. In order to establish and innovate appropriate management strategies, further understanding of the physiological aspects involved in seed dormancy and germination are warranted. In this sense, transcriptomics facilitates the study and identification of molecular mechanisms of interest, by evidencing the activation and repression of genes involved in specific physiological processes. We performed an analysis of the gene expression profiles of *C. odorata* seeds during the transition from the end of dormancy to the onset of germination, by comparing total transcripts present in seeds that are dormant, imbibed to 95 % hydration, and post-germinated. Transcriptome analysis based on a de novo assembly show the differential expression of at least 6,902 genes between dormant and hydrated seeds; of 9,140 genes between dormant and germinated seeds; and 3,734 genes between hydrated and germinated seeds. To our knowledge, this is the first study to address the genetic mechanisms involved in the physiology of dormancy and germination of *C. odorata* seeds and represents the groundwork for further studies of this process in this species

Analysis of promoter activity of the human gene *RB1* in hepatic cancer cell lines by epigenetic drugs.

Jorge Eduardo Vargas Gómez, Paulina Janeth Barraza Reyna, Alain Jesús Aguirre Vázquez, Vianey González Villasana, Mario Abelardo Bermúdez de León.
Centro de Investigación Biomédica del Noreste del Instituto Mexicano del Seguro Social. Dirección: Calle 2 de abril 501, Col. Independencia, Monterrey, N.L., C.P. 64720 Teléfono: 01 (81) 8190-4036. Correo electrónico: distro71@yahoo.com

It has been reported that the tumor suppressor gene *RB1* is repressed in hepatocellular carcinoma. Previously, we have observed that *RB1* mRNA levels were increased in the presence of two epigenetic drugs in the liver cancer cell line HepG2. The possibility to increase RB1 expression in cancer cells could be helpful to combined treatments against hepatocarcinoma. The aim of this work is to analyze the effect of these drugs on *RB1* promoter activity, and to evaluate if these changes are mediated by alterations in the methylation patterns of the *RB1* gene promoter in hepatic cancer cell lines.

Objective: Analyze the promoter activity of the human gene *RB1* in hepatic cancer cell lines by two epigenetic drugs.

Methods. The evaluation of the *RB1* promoter activity was performed by co-transfection in cell lines HepG2 and Huh-7 with the pGL4.10 vector [luc2], harboring the *RB1* gene promoter, and the phRL vector –CMV using policationic lipids. The transfected cells were exposed to epigenetic drugs for 24 h. The evaluation of the methylation patterns in the *RB1* promoter region will be performed by Bisulfite Sequencing PCR in cells exposed to two epigenetic drugs.

Results: Plasmid constructs pGL4.10 [Luc2] and pGL4-RB1 were characterized by restriction enzymes *SmaI* and *MscI*. Sequencing of the *RB1* promoter insert in the pGL4-RB1 vector was performed. Once the plasmids were verified, they were co-transfected in independent assays with the plasmid phRL-CMV in the Huh-7 cell line. Cells transfected with pGL4-RB1 showed higher luciferase activity compared to those transfected with empty vector pGL4.10 [Luc2].

Conclusion: The RB1 promoter is active in hepatocarcinoma cells. Assays with epigenetic drugs are currently in progress.

Deletion of a gene encoding for a chromatin remodeling protein causes increased stress sensibility and reduction of virulence in the phytopathogenic fungus *Ustilago maydis*

Nubia Andrea Villota-Salazar[1], Artemio Mendoza-Mendoza[2], Juan Manuel González-Prieto[1*].
[1*]Laboratorio de Biotecnología Vegetal, Centro de Biotecnología Genómica, Instituto Politécnico Nacional. Blvd. del Maestro esq. Elías Piña s/n, Col. Narciso Mendoza. C.P. 88710. Reynosa, Tam. México. e-mail: jmgonzalezp@ipn.mx
[2] Fungal Molecular Genetics Laboratory, Bio-protection Research Center, Lincoln University, Canterbury, New Zealand.

Chromatin remodeling proteins are important protagonist of the gene regulation processes in eukaryotes. These proteins are responsible for the structural and functional changes in chromatin that epigenetically control the cellular differentiation events in the organisms. Fungi are considered excellent models for the study of epigenetic phenomena and *Ustilago maydis* is one of the most used fungi in research. *U. maydis* is a biotrophic basidiomycete that infects maize (*Zea mays* ssp. *mays*) causing smut disease or ¨huitlacoche¨. This disease is known for the emergence of galls in the infected host tissues, which contain inside of them masses of teliospores of dark appearance. *U. maydis* goes through remarkable morphological and genetic changes to complete its life cycle, and these changes are under epigenetic control.

We deleted a gene corresponding to a chromatin remodeling protein in the genome of *U. maydis* by the PCR based system coupled to *Sfi* I restriction sites, proposed by Kämper (2004). Mutant strain OW4 (*a1b1Δhmt1*) was identified by Southern blot. The sexual partner OW1 (*a2b2Δhmt1*) was obtained from galls after inoculation of maize plants with a mixture of OW4 (*a1b1Δhmt1*) and FB2 (*a2b2*). Morphological observations of OW4 (*a1b1Δkmt1*) and OW1 (*a2b2Δhmt1*) revealed major alterations in size, shape and pigmentation of colonies, as well as in cellular morphology. Tests for the evaluation of the sensibility to osmotic and oxidative stresses in the mutants evidenced, in both cases, increased susceptibility with respect to wild type strains. Pathogenicity assays in greenhouse showed that the virulence of the fungus was affected by the mutation. There was found a higher number of plants with no symptoms of disease in the treatments with the mutant strains than in controls. The deleted gene is involved in the vegetative growth, stress sensibility and virulence of *U. maydis*.

Investigating the role of PARP-1 in the early embryogenesis in *Drosophila Melanogaster*

Jessica Samantha Cruz Ruiz and Mario Zurita.
Department of Developmental Genetics and Molecular Physiology, Institute of Biotechnology, UNAM.
marioz@ibt.unam.mx

The early stages of *Drosophila melanogaster* embryogenesis are characterized by the formation of a syncytium, in which the first rapid 13 nuclear divisions occur without cytokinesis (1-3). During this process, the embryo relies on the maternal genetic material deposited in the egg, until it reaches the mid-blastula transition at cycle 14, when the majority of the maternal products are degraded and the zygotic transcription activation takes place (1-3). There are many different proteins deposited maternally that are involved in the regulation of the first stages of the embryogenesis, as well as they indicate the moment in which the zygotic transcription begins and the correct timing for the maternal products degradation (1-3).

Poly(ADP-ribose) polymerase-1 (PARP-1) is a highly conserved protein, belonging to the PARP family, that catalyzes the synthesis of ADP polymers, which can be attached to different target proteins (4,5). The main functions in which PARP-1 is involved are DNA repair, transcriptional regulation and chromatin structure regulation (4-6). In *D. melanogaster* , there are only two proteins that belong to the PARP family: tankyrase and PARP-1 (4,7). In the fly, hypomorphic mutants of PARP-1 are lethal, and according to RNA-seq data published in *Flybase* , PARP-1 transcript is more abundant in the ovaries and in early embryos, indicating that it is a maternal product (7).

Since PARP-1 is highly involved in transcriptional regulation and it is deposited maternally in the egg, we hypothesize that PARP-1 has an important role in the early embryogenesis in *D. melanogaster* , during the maternal to zygotic transition. To investigate its specific role, we propose three different approaches: 1) Analyze the dynamics of PARP-1-YFP during the early stages of development using confocal microscopy, 2) Observe the phenotype of *D. melanogaster* embryos with lower levels of the maternal transcript using RNAi, and 3) Assess the effect of a specific inhibitor of PARP-1, olaparib, in early embryos.

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Relevance of the protein *N*-linked glycosylation in the virulence and immune recognition of *Sporothrix schenckii*

Nancy E. Lozoya-Pérez., Luz A. López-Ramírez and Héctor M. Mora-Montes*.

Departamento de Biología, DCNyE, Campus Guanajuato, Universidad de Guanajuato, Noria Alta s/n., Col. Noria Alta, Guanajuato, Gto., México. C.P 36050. Tel (473) 7320006 ext 8154, *hmora@.ugto.mx.

S. schenckii is a patogen, dimorphic fungi (yeast and micelial morphotype). Is the etiological agent of sporotricosis which affects animals and humans. In the fungi the cell wall play a important role during host-fungus interaction. To get insights into the role of N-linked glycans during the *S. schenckii*-host interaction we silenced OCH1 and characterized the phenotype of mutant cells, with an emphasis in the interaction with human PBMCs and human monocyte-derived macrophages. Moreover, fungal virulence was analyzed in the *Galleria mellonella*.

The *OCH1* gene from *S. schenckii* was isolated, and the ORF spans 1192bp and encodes for a membrane protein type II of 398aa, with an estimated molecular weight of 44kDa. This gene was expressed in a *Saccharomyces cerevisiae och1*Δ null mutant. The expression of the heterologous gene restored the cell ability to bind Alcian Blue and the sensistivy to cell wall perturbing agents to wild-type levels, suggesting that this gene could be the functional ortholog of *S. cerevisiae OCH1*.

Next, we obtained 5 *S. schenckii* transformants: Ssoch1-10, Ssoch1-2.2, Ssoch1-3.4, Ssoch1-3.7 and Ssoch1-3.21, with 86%, 42%, 98.5%, 100% and 100% of *OCH1* silencing respectively. The number of copies inserted of the construction was determined: Ssoch1-10 1.94 ± 0.43, Ssoch1-2.2 1.89 ± 0.09, Ssoch1-3.4 2.29 ± 0.04, Ssoch1-3.7 1.97 ± 0.05 and Ssoch1-3.21 1.68 ± 0.44.

We observed differences in the amount of rhamnose, glucosamine, and glucose in the cell wall of strains Ssoch1-10, Ssoch1-3.7 and Ssoch1-3.21 when compared to the wild-type (WT) strain. The alcian blue binding assay showed for the WT strain 112.72 ± 4.59 units (µg of dye bound by cells at a DO_{600nm}=1) and the strains that showed a significant difference were Ssoch1-10 (79.91 ± 5.51 units) and Ssoch1-3.7 (72.94 ± 14.87 units). These results suggest that there is probably a change in the cell wall conformation in these transformants.

The silenced strains showed virulence attenuation and a lower ability to stimulate the production of TNFα and IL-6 by human peripheral blood mononuclear cells. The ability to uptake these mutant cells by human macrophages showed to be dependant on the *OCH1* expression.

The use of molecular and genetic tools, such as genetic transformation systems for silencing and inserting or removing genes, proved to be useful to establish the relevance of OCH1 in the *S. schenckii*-host interaction.

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Evaluation of enzymes of clinical relevance in serum and hepatic tissue after abdominal surgery in rats

Violeta Aburto Luna[1], Stephany Hernández Teotan[1], Eduardo Miguel Brambila Colombres[1], Samuel Treviño Mora[1]
[1] Laboratorio de Investigaciones Químico Clínicas, Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla. Av. San Claudio, Col. San Manuel, Puebla Pue., México C.P. 72570. Teléfono: +52 (222) 2295500 Ext. 7483. Correo: samuel_trevino@hotmail.com

Inflammation is an immune response that is activated by various stimuli, such as surgery, producing a series of changes that are mainly regulated by IL-6 and IL-1, which stimulate the hepatic synthesizes of acute phase proteins. On the other hand, the liver also produces diverse enzymes of clinical relevance. Therefore, it is important to evaluate how these enzymes are found after performing abdominal surgery in rats, with and without inhibition of IL-6 and IL-1 signaling. The rats were sacrificed at 3, 6, 9 12, 16, 20 and 24 hours post-surgery. The signaling inhibitors were administered before the surgery and the rats were sacrificed 9 h after surgery. The measurement of the enzymatic activity of ALT, AST, ALP, GGT and LDH in serum and liver was performed according to the Spinreact protocols. To check the inhibition of IL-6 and IL-1 signaling, a Western blot was performed. The surgical trauma produced a serum increase in the enzymatic activity of ALT, AST, ALP, and GGT at all times evaluated, maximum peak registered was at 9 and 12 hours post-surgery. The enzymatic activity in the liver showed a similar behavior, with exception of GGT, which showed no changes. Hepatic ALP activity decreased at inhibiting JAK2 and NFκB signaling. Inhibition of PI3K increases ALP activity. However, PI3K, JNK, and p38 inhibitors decrease ALT, AST, and LDH activity. On the other hand, serum activity of AST, it decreased with the administration of the inhibitors NFκB and PI3K. The inhibitors of JAK2 and STAT3 decreased the AST, ALP and GGT activity. JNK and p38 inhibition increased the AST activity, but decrease ALP and GGT activities. These results demonstrate that increase of IL-1 and IL-6 during a surgical process trigger significant changes in the activity of enzymes of clinical importance, that do not are related to any pathology and that nevertheless, could be used as the follow-up of patient improvement.

Genetic characterization of T cell and B cell receptors of California sea lion

Aranzazu Arias-Rojas, Karina A. Acevedo-Whitehouse*
Laboratorio de Plasticidad Inmune y Ecoepidemilogía Molecular, Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro.
Carretera a Chichimequillas S/N, Ejido Bolaños, 76140 Santiago de Querétaro, Querétaro.
kacevedow@gmail.com
(442) 192-12-00. Ext: 65334

The B (BCR) and T (TCR) cell receptors, control B and T lymphocyte development, survival and activation by biochemical signaling, following antigen recognition in the extracellular environment. Given their functional importance, understanding the structure and evolutionary history of BCR and TCR can provide valuable information the natural history of a species, and, indirectly, shed light on its past pathogenic challenges. However, the study of these receptors is currently focused on biomedical research and its applications, rather than the study of these receptors in the evolutionary context. Our study aims to understand how evolution has shaped the genetics of BCR and TCR receptors of the California sea lion (*Zalophus californianus*). We characterized BCR and TCR receptor chains by means of a comparative and *in silico* analysis. We identified VDJ genetic segments that are highly conserved between carnivorous mammals, and some phylogenetically distal segments (PDS). Interestingly, our preliminary findings show that PDS are important for the interaction between MHC II and TCR in different species. Based on our data, we suggest that PDS of *Z. californianus* BCR could contribute to B cell development. Our molecular, phylogenetic and bioinformatics approach could help answer questions relevant to this non-traditional model species, such as its high tolerance to intestinal parasitic infections or the development of urogenital cancer, a common pathology of this species, which could be relevant to the biomedical field in the future. The study of VDJ segments of lymphocytes receptors in *Z. californianus* could provide insights on the immune response of carnivorous mammals from marine environments.

Study of the death induced by *E. histolytica* trophozoites to different cell lines and its correlation with amebopore

Barrón Oscoy Yurubi[1], González-Canto Augusto[1], Salaiza Suazo Norma[1], Pérez Vázquez Efrén[2], Manjarrez Hernández Ángel[3], López Vancell Rosario[1], Jarillo-Quijada Dolores[1], Pérez Tamayo Ruy[1]. E-mail: yurubibarron@hotmail.com
[1]Research Unit in Experimental Medicine, Medicine Faculty UNAM, [2]Chemistry Engineering Faculty, UNAM & [3] Public Health Department, Medicine Faculty , UNAM. Mexico City, Mexico.

Amebiasis in humans is a parasitic disease caused by *Entamoeba histolytica*, which infectious form is the trophozoite, this parasite is capable to destroy a number of different types of cells such as: neutrophils, erythrocytes and a number of cell lines in culture. One of the molecules from ameba that participates in cell death is the amebopore. This peptide, with a molecular weight of just 8 kDa, is capable of forming pores at the target cell´s membrane and has been implicated in the parasite's cytotoxicity.

One of the aims of this study was to determine the susceptibility of a series of different cells to death induced by amebas. The cells studied were:line A549, Hep-2, HeLa, Vero, Raw, HepG2, peritoneal macrophages and bone marrow macrophages. For doing so, cells were coincubated at 37 °C with trophozoites at different proportions (1:10, 1:25 and 1:50 ameba:cell) during 1, 2 or 3 hours. The viability of cells was determined by Tripan Blue and expressed as percentage of deathcells from the total counted. On the other hand, we analyzed by immunocitochemistry the presence of amebopore at the membrane of those target cells that had been coincubated at 37°C during one and a half hour with trophozoites in a proportion of 1:10 ameba:cell.

Our results show that under the same conditions, from all the different type of cells analyzed, three of them: A549, RAW and bone marrow macrophges were the most susceptible (50% of mortality) to the cytopathic effect of amebic trophozoites. So, at first glance, it doesn't seem to be a direct correlation between the susceptibility observed with the species nor with the origin of the tissue from which the cells proceed.

On the other hand, it's worth underlining that the immunocitochemistry analysis showed that amebopore is associated to the plasmatic membrane of all the cells under study, which indicates that there doesn't seem to exist a correlation either between the susceptibility to the cyopathic effect of trophozoites and the association of amebopore to the membrane of the cells under study.

Low density neutrophils from healthy donors display an activated phenotype

José Carlos Blanco Camarillo and Carlos Rosales
Instituto de investigaciones Biomédicas, UNAM
Email: carlosblanco@ciencias.unam.mx

Neutrophils, also known as polymorphonuclear cells (PMN), are the most abundant leukocytes in human blood and comprise around 50%-70%. PMN are the first cells to migrate into sites of infection or inflammation, in order to eliminate microbial pathogens. The antimicrobial mechanisms of neutrophils include phagocytosis, dragranulation, production of reactive oxygen species (ROS), and the release of neutrophil extracellular traps (NETs). Traditionally, neutrophils are purified from blood by a density gradient with Ficoll. In this method, neutrophils appear at the bottom of the gradient, while mononuclear cells (MNC) appear above in a lower density layer. However, recent reports indicate that in cancer mouse models or cancer patients, there are neutrophils that co-purify with MNC in the low density part of the gradient. These neutrophils have been named “low-density neutrophils”. These low-density neutrophils are not exclusive of cancer, since they have been also found in certain pathologies such as systemic lupus erythematosus, rheumatoid arthritis, psoriasis, HIV infección, malaria, and tuberculosis. It is not known whether these low-density neutrophils are found in sick patients or are also present in healthy individuals. In order to determine the presence of low-density neutrophils in healthy people, and to evaluate their functional phenotype, neutrophils were purified by density gradient and the MNC layer was analysed for the presence of neutrophils. Low-density neutrophils were found in healthy donors, comprising around 10 % of all cells in the MNC layer. The phagocytic activity and capacity to produce reactive oxygen species (ROS) was measured by flow cytometry. Low-density neutrophils when stimulated with PMA produced more ROS than classic (normal density) neutrophils. Also, low-density neutrophils were able to phogocytize antibody-opsonized particles much more efficiently than classic neutrophils. These data suggest that low-density neutrophils in healthy individuals are a subpopulation on neutrophils with an activated phenotype.

Molecular characterization of a rich cysteine protein from *Anopheles albimanus*: its possible involvement during the mosquito immune response.

Victor M. J. Cardoso-Jaime[1,2], Jesús Martínez-Barnetche[1],Humberto Valdovinos-Torres[1], Helena Kuri Magaña[1], Víctor Tsutsumi[2], Salvador Hernández-Martínez[1].

[1]Centro de Investigación Sobre Enfermedades Infecciosas INSP, Cuernavaca Mor. México, C.P. 62100. [2]Centro de Investigación y de Estudios Avanzados del IPN, Ciudad de México, C.P. 07360. E-mail: shernand@insp.mx

Mosquitoes have an efficient immune system able to neutralize pathogens. Mosquito immune system includes humoral (mainly represented by production of antimicrobial peptides, AMPs) and cell-mediated responses (phagocytosis, nodule formation, etc.). Humoral-response is mainly mediated by the fat body and cell-response by hemocytes. Both, hemocytes and AMPs circulate in hemolymph, to reach the whole mosquito body. The hemolymph flow is regulated by the heart, which is the abdominal section of mosquito dorsal vessel. In the mosquito heart are present a pair valves by each abdominal segment, called ostias, through which hemolymph flow into the heart. Two pairs of pericardial cells (PCs, also called nephrocytes because their filtering capacity) are surrounding each ostia. Recently, we reported antimicrobial properties in PCs, suggesting a possible role during the mosquito immune response. In addition, the mosquito heart transcriptome (zymosan challenged) showed up-regulation of AMPs, Toll and IMD components. Interestingly, the most up-regulated gene encode a six cysteine-containing protein, which can form disulfide bridges, a common characteristic of some AMPs. Bioinformatics analyses using an Antimicrobial Data Base, suggest to this protein a high probability to be an AMP. In addition, the cysteines are commons in Factor Von Willebrand domains, which are present on some related proteins with antiviral responses. Interestingly, in mosquito phylogeny, this gene is present only in some anophelines; this genus is easily infected whit parasites but no with virus. The results obtained until now, showed a constant expression trough of mosquito ontogeny, and specifically in thorax, fat body, midgut, and heart from adults. However, by using a polyclonal rabbit antibody against this protein; immunofluorescence assays in challenged mosquitoes showed the protein to be present specifically in hemocytes and PCs. The qRT-PCR and Western Blot analysis, showed over expression of the protein in zymosan-challenged mosquitoes. The present results suggest a possible participation of this protein during the mosquito immune response.

Levels of 17β-oestradiol and testosterone during the infection with *P. berghei* ANKA.

Luis Antonio Cervantes-Candelas, Fidel Orlando Buendía-Gonzales and Martha Legorreta-Herrera. Laboratorio de Inmunología Molecular, Facultad de Estudios Superiores Zaragoza, Campus II, UNAM. Batalla 5 de mayo s/n esq. Fuerte de Loreto, Col. Ejército de Oriente, Iztapalapa C.P. 09230, Ciudad de México. (044) 5547159006. cervantescandelasluis@gmail.com

Introduction: Malaria is a parasitic disease caused by *Plasmodium*, in 2016 it generated 216 million new cases and 445 thousand of deaths (1). The incidence between sexes is the same, but severity of symptoms and mortality is greater in men than in women. Given that the main physiological differences between the sexes are consequence of 17β-oestradiol and testosterone levels and the interaction with their receptors, we analyse whether 17β-estradiol and testosterone are responsible for the sexual dimorphism of malaria. There is a close relationship between the endocrine system and the immune system, since macrophages, dendritic cells, NK cells, T and B lymphocytes, show alpha estrogen receptors (ER-α), whereas the androgen receptors (AR) are presented by T, B lymphocytes and macrophages. In our research group we demonstrated that the decrease in the concentration of sex hormones by gonadectomy modulated the immune response in mice infected with *P. berghei* ANKA (2), in this work we evaluated the effect of sex hormones on the parasitaemia of mice infected with *P. berghei* ANKA.

Methodology: Groups of male and female mice were allocated into: intact, or gonadectomized groups. Half of mice in each group was treated with either 17β-estradiol (545µg / kg of body weight), or with vehicle. Mice were infected with *P. berghei* ANKA and daily parasitemia was measured in Giemsa stained blood films starting on day 3 post-infection. Mice were sacrificed on day 8 post-infection and serum was obtained to evaluate the estradiol and testosterone concentration by EIA. **Results:** The administration of estradiol to male mice increased the parasitaemia on days 6 and 7 post infection compared with the group of intact male mice treated with vehicle, interestingly in this group the levels of oestradiol decreased, but testosterone levels increased. These findings suggest that the decreased levels of estradiol inhibited the adequate activation of cells of the immune response and therefore the parasitaemia decreased. Gonadectomized female mice treated with vehicle or estradiol increased parasitemia compared with gonadectomized males treated with vehicle or with 17□-oestradiol on day 8 post-infection; however, the serum concentration of estradiol and testosterone did not change, suggesting that estradiol and its site synthesis modulates the immune response.

Conclusion: The results suggest that 17β-estradiol promotes parasite elimination and may be involved in the activation of cells of the immune system.

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Effect of *Cymbopogon citratus* and *Artemisia mexicana* on the expression of IL1β in mice infected with *P. berghei* ANKA.

López-Padilla MS1. Legorreta-Herrera M[1].
[1]Laboratorio de Inmunología Molecular, Facultad de Estudios Superiores Zaragoza, UNAM., Batalla 5 de mayo S/N, Col. Ejército de Oriente, Delegación Iztapalapa, Ciudad de México, CP. 09230, Teléfono: 56-23-07-36, Correo electrónico: alcume_100@hotmail.com

Introduction

Malaria is a disease caused by the parasite *Plasmodium*, it is a global health problem, in 2016 the WHO reported 216 million new cases and 445,000 deaths (1). The efficacy of drugs is limited by the generation of *P. falciparum* drug resistance (2). This has motivated the development of different strategies such as the use of plants because their multiple components represent an alternative, since they interact synergistically and delay the generation of resistance (2). In this work, we studied the antimalarial and immunomodulatory properties of *Cymbopogon citratus* and *Artemisia mexicana.* We assessed their effect on the expression of IL-1β, which is involved in the main complication of the disease (cerebral malaria) in CBA/Ca mice infected with *P. berghei* ANKA.

Methodology

We used eight groups of 5 CBA/Ca mice treated as follows: group 1 sterile drinking water, group 2 *C. citratus*, group 3 *A. mexicana* and group 4 chloroquine for 4 days prior to infection. Another 4 groups of mice administered in the same manner not infected were used as controls. On day 8 post-infection mice were sacrificed, and the spleen and brain were isolated. RNA was extracted and retrotranscribed to obtain cDNA, which was amplified by real-time PCR for the IL-1 gene and relative expression was determined compared to β-actin.

Results and Discussion

The administration of *C. citratus* and *A. mexicana* decreased the mRNA expression of IL-1β in the spleen of infected mice. Interestingly, the infection decreased the mRNA expression of IL-1β in the brain of both vehicle-treated mice and mice treated with plants. The administration of *A. mexicana* significantly upregulated the mRNA expression of IL-1β in the brain of no infected mice. Both plants have compounds with anti-inflammatory properties that negatively modulate the expression of this cytokine, but unlike the spleen, a powerful compensatory anti-Inflammatory response is triggered in the brain, resulting in less expression in infected mice than in no infected mice. A possible explanation about the upregulation of IL-1β in the brain of no infected mice treated with *A. mexicana* is the compound Thujone, a ketone with neurotoxic properties that could induce a pro-inflammatory response.

Conclusion

A. mexicana and *C. citratus* negatively modulated the mRNA expression of IL-1β in CBA/Ca infected with *P. berghei* ANKA.

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"Analysis of the expression of CD163 and TWEAK in macrophages and dendritic cells of patients with acute myocardial infarction and its correlation with cardiac function"

Área de Investigación: Inmunidad y Parasitología
Laura Sherell Marín Jauregui, Adriana Elizabeth Monsiváis Urenda, Roberto Fidencio González Amaro, Carlos David Escobedo-Uribe, Alethia Villaseñor.
Centro de Investigación en Ciencias de la Salud y Biomedicina (CICSaB), Facultad de Medicina, UASLP. Departamento de Cardiología, Hospital Central Dr. Ignacio Morones Prieto.
Dra. Adriana E. Monsiváis Urenda
Centro de Investigación en Ciencias de la Salud y Biomedicina
Av. Sierra Leona No. 550, Fracc. Lomas 2ª Sección, C.P. 78210 Teléfono 444 8261450 ext. 8234
aurenda@gmail.com

Keywords: Myocardial infarction, heart failure, TWEAK, CD163.

Abstract

Backgrownd. Cardiovascular diseases are the leading cause of death worldwide. Coronary Artery Disease (CAD) is characterized by partial or total obstruction of the coronary arteries by atheromatous plaque, which, due to its growth, can trigger an acute myocardial infarction (AMI). After AMI, changes in cardiac tissue can develop pathological remodeling, which triggers ventricular failure. It has been described that CAD can be the underlying cause of Heart Failure (IC). Different cellular populations participate in the development of this pathological entity, such as dendritic cells and macrophages, as well as pro-inflammatory cytokines. Paradoxically, clinical trials for the neutralization of pro-inflammatory cytokines such as Tumor Necrosis Factor (TNF) show no therapeutic benefits, rejecting the complex network of cytokines participating in HF.

Objective. To evaluate the expression of CD163 and TWEAK in myeloid dendritic cells (mDC) and plasmacytoid cells (pDC), as well as in macrophages of patients with AMI, and to correlate these parameters with ventricular function at 3 and 6 months after AMI.

Methods. Patients with a diagnosis of AMI (n = 30) who received reperfusion therapy were recruited. An echocardiogram was performed to evaluate the the left ventricle ejection fraction (LVEF), after which a peripheral venous blood sample was taken 72 hours post-infarction, as well as at 3 and 6 months later. Peripheral blood mononuclear cells (PBMC) and plasma were obtained. A PBMC fraction were subjected to surface labeling with antibodies coupled to fluorochromes, to measure the expression of CD163 and TWEAK in mDC and pDC. The remaining fraction was subjected to positive selection for the isolation of monocytes, cultured for the differentiation of macrophages M1 and M2 and subsequently the expression of TWEAK (M1) and CD163 (M2) was measured by multicolored flow cytometry.

Results. 23 patients with a diagnosis of AMI have been recruited, and 11 patients have been followed up at 3 months, and 5 patients at 6 months post-infarction. Preliminary data analysis shows no significant differences in levels of TWEAK and CD163 in both dendritic and macrophage cells. However, the recruitment of subjects with AMI is still performed, as well as the follow-ups for their subsequent correlation analysis.

Insight about the lethal effect of curcumin on *Taenia crassiceps* cysticerci

José de Jesús Martínez-González, Alberto Guevara-Flores, Juan Luis Rendón
e Irene Patricia del Arenal Mena*.
Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México.
Correspondencia: Facultad de Medicina, Ciudad Universitaria; Ciudad de México,
CP 04510 Tel. +52(55) 5623-2169 *Correo electrónico: darenal@bq.unam.mx

Curcumin (also named diferuloylmethane) is a hydrophobic molecule obtained from the rhizome of turmeric (*Curcuma longa*) which has been widely studied due to its antioxidative, antitumoral and antiparasitic properties. As regards the latter, curcumin has been successfully used against the parasitic protozoa *Leishmania major*, *Trypanosoma brucei*, *Giardia lamblia*, and *Plasmodium falciparum,* as well as in nematode *Setaria cervi*, showing a lethal effect in all of them. However, the mechanism underlying such anti parasite effects has not been characterized.

In this work, the effect of curcumin on the larval state of the cestode *Taenia crassiceps* is analyzed. The results revealed curcumin is highly toxic to the parasite. The effect of the compound was dose-dependent. At curcumin concentrations higher than 500 micromolar, a mortality rate of 100% on cysticerci population was reached. The lethal effect was observed two hours after addition of the compound.

In order to gain insight in the molecular origin of the lethal effect of curcumin, the production of ROS, as well as the residual activity of the enzyme thioredoxin glutathione reductase (TGR) was measured in crude extracts of whole cysticerci. Following curcumin administration, a dramatical increase in ROS production was observed, in spite of a low inhibition of TGR. Incubation of cysticerci with the antioxidant compound N-acetyl cysteine (NAC) only slightly revert ROS production.

Hence, we suggest the lethal effect of curcumin in *Taenia crassiceps* cysticerci occur through pleiotropic mechanisms.

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Anti-*Trichomonas vaginalis* effect of tritrpticin-derived peptides

Moisés Adrián Martínez-Padilla[1], Marco Antonio Barajas-Mendiola[1], Julia Jaquelina Guzmán-Rodríguez[2], Alejandra Ochoa-Zarzosa[3], Joel Edmundo López-Meza[3], Ruth Reyes-Cortés[1], Eva Edilia Ávila-Muro[1], Patricia Nayeli Alva-Murillo[1]
[1]Departamento de Biología, DCNE, Campus Guanajuato, Universidad de Guanajuato, Noria Alta S/N, C.P. 36050, Guanajuato, Gto. Tel (+52) 473 732 006 Ext. 8199. pn.alva@ugto.mx
[2]Departamento de Medicina Veterinaria y Zootecnia, DICIVA, Campus Irapuato-Salamanca, Universidad de Guanajuato.
[3]Centro Multidisciplinario de Estudios en Biotecnología-FMVZ, Posta Veterinaria, UMSNH, Morelia, Michoacán.

Trichomoniasis is the main common non-viral sexually transmitted infection worldwide, and its etiological agent is the parasite *Trichomonas vaginalis*. Some health concern related to trichomoniasis are (i) the asymptomatic condition of carrier men and the chronic inflammation in women, (ii) its predisposing nature to other diseases (i.e. HIV infection, prostate and cervical cancer) or consequences (premature births), and (iii) parasite resistance to classical treatment (metronidazole). For these reasons, the exploration of new alternative treatments is essential. In this way, antimicrobial peptides (AMPs) are attractive candidates as treatments; however, there are some disadvantages, such as hemolytic activity. Design and *in silico* improvement of AMPs-derived peptides are innovative bioinformatic tools that allow optimizing the AMPs features (i.e. higher parasitic effect or lower cytotoxic activity). Few studies are focused on the effect of AMPs and/or their derivatives against *T. vaginalis*. In this sense, porcine cathelicidin-derived tritrpticin (VRRFPWWWPFLRR) has trichomonacide effect but shown hemolytic activity. Therefore, tritrpticin is an attractive molecule for improving its characteristics. The goal of this study is to design tritrpticin-derived peptides (TDP) with the aim of reduce the hemolytic activity and maintain its anti-*T. vaginalis* effect. For this, we analyzed the primary sequence of tritrpticin with HemoPI (http://crdd.osdd.net/raghava/hemopi/), a web server for computing hemolytic potency peptides, and the TDP with PROB score close to 0 -as the lowest putative hemolytic activity- were selected. The results of this *in silico* analysis showed a PROB score of 0.66 for tritrpticin, and two primary sequences of TDP with PROB score of 0.17 (VRRFAWWWPFLRR and VRRFPYYYPFLRR) were selected. Peptides were manually synthetized (0.05 mmol scale) following Fmoc solid phase peptide standard protocol. The analysis and purification of peptides is in process using HPLC. Identities of peptides will be confirmed by mass spectrometry analysis. Then, the antiprotozoal and hemolytic activities of TDP will be tested by trypan blue exclusion and hemolytic assays, respectively. We expect that

The thioredoxin reductase from *Entamoeba histolytica*as a promising target against amoebiasis disease

[1]Yoalli Martínez Pérez, [1]Mario NequizAvedaño, [1]Marco E. Gudiño Zayas, [1]Edith Mendoza Tenorio, [3]Emma Saavedra Lira, [1]Ruy PérezTamayo, [2]Ithzel García Torres, [2]Sergio Enríquez Flores,[1]Alfonso Olivos García.
[1]Unidad de Investigación en Medicina Experimental, Facultad de Medicina, UNAM. [2]Grupo de Estudio en Biomoléculas y Salud Infantil, Laboratorio de EIMyT, Instituto Nacional de Pediatría. [3]Departamento de Bioquímica, Instituto Nacional de Cardiología, Ignacio Chávez.
Contact: AOG, mail: olivosa@yahoo.com, phone: 56232663

Entamoeba histolytica is an intestinal protozoan that causes amoebic colitis and systemic amoebiasis of the liver, lung and brain. This intestinal illness is responsible for 100,000 deaths year worldwide affecting mainly underdeveloped countries with poor sanity conditions.During tissue invasion, *E. histolytica* is exposed to a high concentration of oxygen and reactive oxygen species (ROS) from inflammation. However, the parasite can balance ROS by antioxidative defense mechanisms, such as NAPH: flavin-oxidoreductase (Eh34) and the thioredoxin reductasa (TrxR) / thioredoxin (Trx) system. The Eh34 catalyzes the dependent reduction of NAPH from O_2 to H_2O_2. The TrxR enzyme has NAPH oxidase activity generating H_2O_2 from the direct reduction of O_2. Also, TrxR has the ability to catalyze the reduction of disulfide compound like Trx and other substrates. In these sense, these proteins play an important role in the REDOX system, which may be critical for the survival and virulence of the parasite exposed to high concentrations of oxygen and ROS. Since cysteines (Cys) may be important for structure and function of many enzymes, we explored new molecules that potentially may chemically modify the Cys of TrxR to promote destabilization. As a first approach to the search for the new compounds, inactivation studies were carried out through the chemical modification of Cys residues. We proved the rabeprazole effect (a proton pump inhibitor) on TrxR activity.

Our results show that rabeprazole is able to inhibit the TrxR activity by 40%. Subsequently, *in vitro* studies were carried out and *E. histolytica* trophozoites were incubated at different times (6, 12, 24 and 72 h) with increasing concentrations of rabeprazole (200, 400 and 600 µM). Rabeprazole either 600 µM in 24 h or 400 µM in 72 h diminished amoebic viability by 50%. In addition, sublethal *in vitro* incubation of amoebae with rabeprazol inhibited the parasite ability to cause amoebic liver abscess in hamsters. The charaterization of new amoebic targets may allow to find new compounds for the treatment of human amoebiasis.

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Cell cycle characterization in the *Anopheles albimanus* midgut cells.

Krystal Maya Maldonado[1,2]. Victor Manuel Jonathan Cardoso Jaime[1,2]. Víctor Hugo Rosales[3]. Fidel de la Cruz Hernadez-Hernandez[1]* Humberto Lanz-Mendoza[2]*
[1]Department of Infectomics and Molecular Pathogenesis, Center for Research and Advanced Studies of the National Polytechnic Institute (CINVESTAV) Mexico City. [2] Center for Infectious Diseases Research, National Institute of Public Health (CISEI-INSP), Cuernavaca Morelos Mexico. [3]Flow Cytometry Unit of General Coordination of Experimental Services (CGSE). National Laboratory of Experimental Services (LaNSE) at Cinvestav Mexico City. 1*cruzcruz@cinvestav.mx
[2]* humberto@insp.mx

In mosquitos the midgut plays an important role limiting the development of *Plasmodium* and other pathogens. This organ is formed by a cell layer that permits the molecular exchange. Three different cell types have been described in the midgut of mosquitoes: endocrine, columnar and regenerative. Columnar and endocrine cells have microvilli exposed to intestinal lumen with the principal function of secreted digestive enzymes and absorb nutrients. The regenerative cells (stem cells) are distributed in the basal side of the midgut epithelia. Cellular division has been observed in the mosquito midgut during injure repair. However, the molecular and cellular mechanisms behind this process is not well understood.

We have reported an adaptive immune response (immunological priming) in the mosquito *An. albimanus.* In this process, an important DNA synthesis *de novo* has been observed in the midgut and it has been hypothesized that this DNA synthesis could be due to an endoreplication process. In this work, with the aim to understand the endoreplication process, we characterize the cell cycle process in the mosquito midgut.

Using bioinformatics tools we identify the sequences of molecules (Cyclins: CycA, CycB, CycE and Cdk2) that can be used as markers of cell cycle progression and primers for PCR and qPCR were designed. DNA amplification was conducted on fifteen mosquito midguts samples at different post-emergency times. During the whole life of these mosquitoes, they were fed with 10% sugar solution added with antibiotic and antifungal (PSN 1x). Cyclin B is a protein that is found in the G2 / M phase of the cell cycle and in this case was detected at 6, 24, 48 h post emergency. On the other hand, phases of the cell cycle at different post-emergency times were evaluated by flow cytometry in disaggregated cells from midgut tissue. Flow cytometry results are similar with the pattern cyclins expression by qRT-PCR that we performed. We have identified that cells in mitosis continue to be present up to 9 days post-emergence in the mosquito midguts. When midgut cells were evaluated after priming induction with *Plasmodium* it was observed that G2 / M phase of the cell cycle disappeared. These results suggest that mitosis is possible in the adult midgut during the first hours post- emergency and in midguts without immune priming induction. Therefore, these results indicate and important cell cycle dynamic during midgut interaction with pathogens. This work is one of the first involving cell cycle characterization in mosquito cells.

Cloning, purification and biochemical characterization of the recombinant Iron-Sulfur Flavoprotein of *Entamoeba histolytica*

[1]Paulina Mejía Del Castillo, [2]Itzhel García Torres,[1]Mario Nequíz Avedaño, [1]Edith Mendoza Tenorio,[3]Emma Saavedra Lira, [1]Ruy Pérez Tamayo, [2]Sergio Enríquez Flores,[1]Alfonso Olivos García
[1]*Unidad de Investigación en Medicina Experimental, Facultad de Medicina, UNAM.* [2]*Grupo de Estudio en Biomoléculas y Salud Infantil, Laboratorio de EIMyT, Instituto Nacional de Pediatría.* [3]*Departamento de Bioquímica, Instituto Nacional de Cardiología, Ignacio Chávez.*
Contact: AOG; mail: olivosa@yahoo.com; Phone: 56232663

Introduction: Amoebiasis is a parasitic disease caused by the protozoan *Entamoeba histolytica*. This illness is responsible for one hundred thousand dead worldwide and in Mexico is the most prevalent parasitosis. *E. histolytica* and the non pathogenic *E. dispar* live and multiply in the human large intestine; however only*E. histolytica* is able toinvade intestine and to cause tissue damage. During such invasion *E. histolytica* has to resist the toxic high oxygen concentrations present in tissues (pO_2 4-16%) and reactive oxygen species from inflammation. To resist them *E. histolytica* has an oxygen reduction pathway (ORP) composed by flavodiiron protein ($O_2 \rightarrow H_2O$), Eh34 protein ($O_2 \rightarrow H_2O_2$) and peroxiredoxin ($H_2O_2 \rightarrow H_2O$). Despite the importance of ORP in amoebic pathogenicity an Iron Sulfur Flavoprotein (ISF), that could be able to detoxify O_2 and H_2O_2, has received poor attention.

Objective: To clone, express and characterize ISF of *E. histolytica*.

Methodology and results:A search of the *EhISF* gene was carried out in the NCBI database. Once this sequence was achieved, it was translated to amino acids to identify the main characteristic of a flavoprotein like enzyme; the cysteine motif. On the other hand, oligonucleotides were designed toperform a PCR by using DNA of *E. histolytica*. Thereafter, the *EhISF* gene was cloned in the plasmid pJET1.2. Then, gene *EhISF* was digested with the restriction enzymes *NdeI* and *BpuI102I*, and ligated in the expression vector, pet3a-HisTEV.This plasmidhasimportant characteristics including a sequence of six histidines that allow protein purificationbyaffinitychromatography with Ni^{2+}.Once this pet3a-HisTEV / EhISF ligation was performed, the EhISF recombinant protein was overexpressed in *Escherichia coli* SHuffle T7strain that grew in LB medium supplemented with ampicillin. The bacterial culture was induced with 0.5 mM IPTG, and the cells were grown for 12 h at 16 °C. The bacteria were harvested by centrifugation and the soluble fraction was applied onto a Ni2+ column and eluted with imidazole. The overexpression and purity of the EhISF protein was verified by SDS-PAGE 16% that showeda band of□23.8 KDa. This is a preliminary study in which for the first time we successfullyoverexpressedthe ISF protein of *E. histolytica*.

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Fecal Lactobacilli Count in BALB/c Mice Treated with Bovine Lactoferrin

[1]Melendez-Avalos Araceli, [2]Sánchez-García Quetzalcóatl, [1]Vega-Bautista Alan, [3]Godínez-Victoria Marycarmen, [3]Campos-Rodríguez Rafael,[1]Drago-Serrano Maria Elisa (dragome@yahoo.com)
[1] Depto. Sistemas Biológicos, UAM-Xochimilco, [2]Depto. Bacteriología, Escuela Nacional de Ciencias Biológicas, IPN, [3] Secc. Estudios de Posgrado e Investigación, Escuela Superior de Medicina, IPN. Working place: Lab Microbiología Molecular, UAMX, Calzada del Hueso No. 1100 CDMX 04960, CDMX, Tel +5255 54837000 ext 3624

Introduction. Intestinal microbiota has a prime role on the microbial antagonism by hampering the growing and colonization of pathogenic organisms favoring the homeostasis. Intestinal microbiota composition is under the endocrine control of sexual hormones. Bovine lactoferrin (bLf) stimulates the growing of indigenous microbiota members like Lactobacilli as documented by culture and molecular assays. Effect of bLf on fecal Lactobacilli load in both genders is unknown **Aim**. To assess the influence of oral bLf on fecal Lactobacilli count in female and male mice **Methods**. Groups (n=4) of 8 week-old BALB/c mice were treated 4 days with either 0.5 % or 5% bLf (female) as well as 4 or 7 days with 0.5 % bLf (male). Water (control group) or bLf were *ad libitum* administered within drinking bottles. On the last day, fecal pellets were collected and weighed from each mouse in microtubes with 0.5 mL of thioglycolate broth. Feces were fully homogenized and 10 fold serial dilutions in thioglycolate broth were prepared and plated in MRS agar. After 48 hr of anaerobic incubation at 37oC colonies were counted and expressed as the colony forming units (CFU) per g feces. Two experiments were done, and data were expressed as mean and standard deviation; comparisons of three or two groups (female *vs* male) were analyzed by one-way ANOVA or Student *t* test, respectively. Significant differences were regarded at $p<0.05$ **Results**. In female mice, fecal lactobacilli count in 0.5% bLf-treated was higher than control and the 5% bLf-treated group. No differences were found between these two latter groups. In male mice, fecal Lactobacilli count in 7-days bLf-treated group was lower than the control group but no differences were found with 4-days bLf treated group **Discussion**. Data suggested that 0.5 % bLf treatment for 4 days may be optimal by increasing (female) or un-affecting (male) the Lactobacilli count. Treatment with 5% bLf by 4 days (female) and with 0.5% bLf for 7-days (male) may be potential inflammatory effect by decreasing the lactobacilli count. Differential effect of bLf on female and mice may reflect an effect of sexual hormones **Perspectives**. To optimize the bLf treatment to explore the modulatory effects on gut homeostasis.

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Anti-*Trichomonas vaginalis* activity of hexanoic extract of avocado seed

Blanca Yessenia Mendez-Grimaldo1, Susana Flores-Gallegos1, Marisol Báez-Magaña2, Alejandra Ochoa-Zarzosa2, Joel Edmundo López-Meza2, Rafael Salgado-Garciglia3, Patricia Nayeli Alva-Murillo1*
1Departamento de Biología, DCNE, Campus Guanajuato, Universidad de Guanajuato, Noria Alta S/N, C.P. 36050, Guanajuato, Gto. Tel (+52) 473 732 006 Ext. 8199. pn.alva@ugto.mx
2Centro Multidisciplinario de Estudios en Biotecnología-FMVZ, Posta Veterinaria, UMSNH, Morelia, Michoacán.
3IIQB, UMSNH, Ciudad Universitaria, Morelia, Michoacán.

The protozoan parasite *Trichomonas vaginalis* (TV) causes trichomoniasis, one of the most common non-viral sexually transmitted infection worldwide, resulting into serious obstetrical and gynecological issues. This pathology is classically treated with the 5-nitroimidazole class drugs (i.e. metronidazole). Studies have shown that clinical cases of trichomoniasis are caused by parasite resistant to metronidazole. Thereby, new approaches to discover natural compounds against TV have been performed, and medicinal plants are an invaluable source. Mexican avocado (*Persea americana* var. drymifolia) is used in Mexican traditional medicine. It is known that chloroformic and ethanolic extract of *P. americana* seeds showed antiprotozoal activity. Additionally, hexanoic extract of Mexican avocado seed (HEMAS) has immunomodulatory properties. However, its anti-TV activity is unknown, which was the aim of this study. For this, two clinical strains of *T. vaginalis* (GT13 and GT21) were used, these strains were isolated from the vaginal secretions of patients with symptomatic trichomoniasis. The antiprotozoal activity of HEMAS (0.25, 0.5 and 1 □g/mL) was tested by trypan blue exclusion assay. Metronidazole (1 µg/mL) was used as control, as well as DMSO 5% due to HEMAS was dissolved in this vehicle. The lethal concentration 50 (LC50) was calculated by Probit analysis. The results showed that DMSO 5% did not alter trophozoites viability at 24 h. Contrary, metronidazole (1 µg/mL, 24 h) diminished parasites viability (59-74%). Interestingly, HEMAS inhibited TV GT13 viability in a concentration-dependent manner (42-75%), and this effect was similar for TV GT21 (17-73%). In TV GT13 strain, 0.1 µg/mL of HEMAS reduced trophozoites viability (74%) even lower than metronidazole (59%). Regarding to LC50, we calculated a LC50 of 0.28 µg/mL for TV GT13 and 0.26 µg/mL for TV GT21. In addition, assays to determine if HEMAS improves the effect of metronidazole on trophozoites as well as the hemolytic activity of HEMAS are in course. In conclusion, HEMAS inhibited TV viability and this effect is independent of the strain, which could be of pharmacological interest.

Recombinant protein harboring relevant epitopes from Influenza A H1N1, as a new candidate vaccine against flu.

Karen Lizbeth Reyes-Barrera[1], Nohemí Salinas-Jazmín[1], Ruth-Elena Soria-Guerra[2], Ángel Gabriel Alpuche-Solís[1*]
[1]División de Biología Molecular (IPICYT), [2] Universidad Autonoma de San Luis Potosí. CP. 78216, Tel: (444) 834 2080,*alpuche@ipicyt.edu.mx

The flu is the main respiratory illness in the world produced by influenza virus.

This virus has evolved in several evasion mechanisms to overcome the immune system, so new pandemic strains can be arisen. For this reason the influenza vaccines require improvements annually. The aim of the current work was to express a multiepitope protein (MP) in *E. coli*, based on the conserved and relevant epitopes from influenza virus A H1N1. By mean of a bioinformatics approach the putative antigenic and immunogenic epitopes from all circulating strains were analyzed. A codon bias optimized synthetic gene was cloned into pET28b plasmid and transformed into the strain *E. coli* BL21. We induced the protein production with 0.5 µM IPTG, then the inclusion bodies were purified and solubilized. We purified the MP by nickel affinity column. The protein fractions were characterized by: Bradford, ELISA and SDS-PAGE assays. The immunization assay performed in BALB/c mice consisted in three experimental groups: 1) Tris; 2) Tris + Freund´s Incomplete Adjuvant (FIA); 3) 5 µg of MP + FIA. A three immunization scheme was designed, one subdermic and two intraperitoneal boosts. The serum of mice analyzed showed the highest IgG titer (1:320,000) in group 3 and none of the groups showed IgE antibodies production.The affinity of the antibody was corroborated by Western Blot assay. The results showed that the polyclonal antibodies obtained in mice after MP immunization, are specific against MP and also that MP is not an allergenic protein. We are conducting *in vitro* tests to demonstrate the ability to neutralize the influenza virus.

Effect of blocking oestrogen receptors on the IFN-γ and IL-10 mRNA expression in the brain of mice infected with *P. berghei* ANKA

Teresita de Jesús Nolasco-Pérez, Luis Antonio Cervantes-Candelas and Martha Legorreta-Herrera. Laboratorio de Inmunología Molecular, Facultad de Estudios Superiores Zaragoza, Campus II, UNAM. Batalla 5 de mayo s/n esq. Fuerte de Loreto, Col. Ejército de Oriente, Iztapalapa C.P. 09230, Ciudad de México. (045)5547611531. teresyta.qfb@gmail.com

Introduction: Malaria is the most severe parasitic disease in the world; it is produced by the parasite *Plasmodium.* In 2016, it caused 445,000 deaths (1). The mortality and severity of malaria is higher in men than in women, it presents sexual dimorphism (2). The most severe complication associated with death is cerebral malaria (CM), which, is associated with an excess of proinflammatory cytokines, such as IFN-ϒ, which can be suppressed by interleukin 10 (IL-10), an anti-inflammatory cytokine. Since the sexual steroids such as 17β-oestradiol levels are responsible of the main differences between both sexes, it is likely that this hormone is responsible for sexual dimorphism. The cells of the immune response have receptors for estradiol, so it is possible that 17β-oestradiol modulates the immune response against *Plasmodium*. In this work, we blocked the oestrogen receptors with tamoxifen to evaluate their effect on the expression of IFN-ϒ and IL-10 in the brain of mice infected with *P. berghei* ANKA.

Methodology: 4 groups of 10 female CBA/Ca mice were treated with tamoxifen (1 mg/kg body weight) or vehicle for 28 consecutive days. A group treated with tamoxifen and one with vehicle were infected with 1x103 *P. berghei* ANKA-parasitized erythrocytes. After 4 days post-infection, parasitemia was evaluated daily by optical microscopy. All groups were sacrificed at day 8 post-infection and mRNA obtained from the brain was extracted, reverse transcripted and the cDNA obtained was used to amplify IFN-ϒ and IL-10 genes using real-time PCR.

Results and discussion: Block estrogen receptors down regulated the mRNA expression of IFN-ϒ and IL-10 compared to the group treated with vehicle, in the brain of female CBA/Ca mice infected with *P. berghei* ANKA. These findings suggest that oestrogens upregulate the synthesis of IFNϒ in the brain only during *Plasmodium* infection such as probably in astrocytes or microglia cells which decreases the synthesis of IFN-ϒ, and therefore IL-10 mRNA expression also decreases.

Conclusion: Oestrogens upregulates the mRNA expression of IFN-ϒ and IL-10 in the brain of mice infected with *P. berghei* ANKA.

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Immunomodulatory effects of thionin Thi2.1 from *Arabidopsis thaliana* on bovine mammary epithelial cells

María T Arceo-Martínez1, Marisol Baez-Magaña1, Violeta Díaz-Murillo1, Joel E. López-Meza1 and Alejandra Ochoa-Zarzosa1*
1 Centro Multidisciplinario de Estudios en Biotecnología, Universidad Michoacana de San Nicolás de Hidalgo. Km 9.5 Carretera Morelia-Zinapécuaro, La Palma. Tarímbaro, Michoacán. CP 58893, México. Tel/Fax: 01(443)295-8029. Autor para correspondencia: aleocho@hotmail.com

Antimicrobial peptides (AMPs) are key elements of plant defense mechanisms,resembling conserved protection strategies also present in mammals. Among the AMPs, plant thionins are particularly interesting due that display antibacterial and antifungal activities. In *Arabidopsis thaliana* have been described four thionins: Thi2.1, Thi2.2, Th2.3 and Thi2.4. Work from our group shows that Thi2.1 expressed by bovine endothelial cells has direct antibacterial activity against *Staphylococcus aureus* mastitis isolates, bacteria able to persist inside bovine mammary epithelial cells (bMECs). Thus, the objective of this work was to analyze the immunomodulatory effects of the antimicrobial peptide thionin Thi2.1 from *A. thaliana* on bMECs during *S. aureus* infection. According to the results, *S. aureus* internalization into bMECs was reduced in cells pre-treated with Thi2.1 at 5 and 10 µg/mL during 24 h, effect related to the participation of TLR2. In addition, bMECs pre-treated with Thi2.1 (24 h) significantly increased TNF-α (~2-fold) and IL-6 (~7- fold), whereas decreased IL-10 gene expression (~0.5-fold). Interestingly, Thi2.1 inhibits the up-regulation induced by *S. aureus* of TNF-α and IL-10 gene expression, as well as nitric oxide production. In addition, Thi2.1 (10 µg/mL) up-regulates the expression of the chemokine IL-8 (~3-fold) in infected bMECs. Some of these effects are related to TLR2 activation. In this sense, Thi2.1 also reduces *S. aureus*-induced TLR2 gene expression and membrane abundance. In conclusion, Thi2.1 from *A. thaliana* modulates bMECs innate immune response by inducing the production of pro- and anti-inflammatory molecules while inhibits *S. aureus* internalization. Some of these effects are mediated by TLR2. This is the first report describing immunomodulatory properties of a plant thionin in mammals.

Biochemical characterization of allergens isolated from different sources for skin tests.

Aranxa Zu Ortiz Pacheco, Patricia Morales Arrieta and Sandra Morales Arrieta
Universidad Politécnica del Estado de Morelos, Boulevard Cuauhnáhuac No. 556 Col Lomas del Texcal Jiutepec Morelos. CP 62550
18070805@upemor.edu.mx 7772293533

INTRODUCTION. In the last 10 years, allergies have had a constant increase in prevalence worldwide, which according to the World Organization of Allergy (WAO) has become an epidemic and a health problem. [1]
Approximately 30-40% of the world population lives with one or several allergic diseases. In Latin America it is speculated that approximately 15% to 23% of the population have some kind of allergy. [2]
Allergy tests have been tested to be effective, but due to patents on allergen extracts procedures in other countries such as Spain, Cuba and Argentina, the cost of tests in Mexico is considered high. Our work is focused on the characterization of new extracts of bacterial allergens, through the application of treatments with wet heat and sonication to pathogenic microorganisms, resulting in the attenuation of these, and thus be able to find a procedure for the production of allergen extracts where it is more feasible and affordable to obtain it in Mexico.
Our objective is to biochemically characterize environmentally isolated microbial allergens as potential use for skin tests.
METHODOLOGY. Microorganisms were isolated from blackberries with the *NOM-114-SSA1-1994*. Molecular identification of the isolated microorganisms was carried out with PCR 16S rRNA and they were sequenced. Each isolated microorganism was characterized by carrying out a growth kinetics in a RTS-1 personal bioreactor and a quantification of biomass by dry weight. The attenuation was carried out in two parts, by temperature beginning with the maximum temperatures of each bacterium and by sonication.
RESULTS. From isolation, two strains were identified, *Salmonella enterica* and *Escherichia coli*, growth kinetics were carried out, obtaining *S. enterica at* the stationary phase starts at 7 h and for *E. coli* at 5.5 h.
In the attenuation with temperature it was observed that there was a decrease of *S. enterica* growth at 65°C and for *E.coli* at 70°C. The attenuation of *S. enterica* with the ultrasound treatment were satisfactory in both bacteria
CONCLUSIONS. *In vitro* skin tests of *S. enterica* and *E. coli* strains attenuated with wet heat and sonication treatments could be another treatment of attenuation different from chemical solutions, which are currently are used.
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Cytokine Profile in Serum and Bronchoalveolar Lavage from Patients with Primary Sjögren's Syndrome

Daniel Paz Gómez, Alfonso Salgado Aguayo, Dalia Ramos González, Miguel Gaxiola Gaxiola y María del Carmen Navarro González

Instituto Nacional de Enfermedades Respiratorias "Ismael Cosío Villegas". Laboratorio de Enfermedades Reumáticas. Calz. de Tlalpan No. 4502, Del. Tlalpan, Col. Sección XVI, Ciudad de México. C.P. 14080. Tel: 54871700 Ext 5187. danielpazgmz@gmail.com

INTRODUCTION. Sjögren's Syndrome (SS) is an autoimmune disease that occurs predominantly in women and is characterized by exocrine gland destruction, resulting in xerophtalmia and xerostomia. Sometimes, other organs such as the lung might also be affected in SS causing diffuse interstitial lung disease (DILD) in 20% of the cases. The physiopathology of SS is not fully understood but it is known that B and T cells play an important role. 75% of infiltrating lymphocytes in exocrine glands are T cells and most of them are helper T (TH) cells. TH cells are further classified depending on the cytokines

they produce, thus the cytokine profile might indicate the subclass of TH cells present.

OBJECTIVE. Analyze the cytokine profile in serum and Bronchoalveolar lavage (BAL) from primary Sjögren's Syndrome (pSS) patients and healthy individuals.

METHODS. Sera and BALs from 14 female and 2 male pSS patients ages 42 to 72 (media of 56.94) were obtained and analyzed using BioPlex Pro Human Cytokine 17-plex Assay (Bio-Rad) from Luminex in a MagPix equipment (ThermoFisher). Sera and BALs from 6 female and 2 male healthy individuals were used as controls. Duplicate test were performed for each sample and t Student were used in the statistical analysis.

RESULTS. There was a different cytokine profile in sera than in BAL in patients with pSS when compared to healthy individuals. Cytokines MCP-1, IL-1β , IL-6, IL-7, IL-8, and IL-10 were enriched in sera from patients with pSS; while there were statistically significant differences in the abundance of IL-6, IL-17, IFNγ, GM-CSF, and G-CSF in BALs from pSS patients.

CONCLUSIONS. The enrichment of TH1 related cytokines in sera from pSS patients (MCP-1, IL-1β, IL-6, IL-7) suggest a predominant TH1 systemic immune response in this disease; however, the increased abundance of IL-17 in BALs might indicate a local TH17 immune response in the lung of patients with pSS

CD13 mediates phagocytosis in human neutrophils

Gloria Erandi Pérez Figueroa, Pablo A. Álvarez Carrasco, Enrique Ortega
Departamento de Inmunología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Ciudad Universitaria, Ciudad de México. México. 562-23834.
ortsoto@biomedicas.unam.mx

Phagocytosis plays a critical role in innate immunity for elimination of pathogens. This process is mediated by different membrane receptors, such as FcγRs or Complement receptors. CD13 is a membrane-bound ectopeptidase, highly expressed on monocytes, macrophages, and neutrophils. In monocytic cells, CD13 is involved in diverse functions, including degradation of peptide mediators, cellular adhesion, migration and modulation of FcγRs- mediated phagocytosis. However, the function of CD13 in neutrophils (one of the first lines of defense against microorganisms) has been less studied. In this work, we studied the ability of CD13 to mediate phagocytosis and the production of reactive oxygen species (ROS), and to modulate Fc□R-mediated phagocytosis and ROS production, in human neutrophils.

Neutrophils were obtained from peripheral blood of healthy donors by density gradient centrifugation. Cells were incubated were incubated with Fab fragments of anti-CD13 mAbs 452 and C, Fab fragments of mAb IV.3 (anti-FcγRII), or with mAb 3G8 (anti-CD16b). Sheep erythrocytes coated with Fab'2 frgaments of goat anti.mouse IgG were added. Phagocytosis was quantified by flow cytometry and ROS production was quantified with the ROS-sensitive fluorescent dye Carboxy-H2DFFDA respectively.

We found that in human neutrophils, CD13 mediates efficient phagocytosis very similary to the one carried out by the FcγRs receptors. Also, we demonstrated that CD13-mediated phagocytosis is coupled to ROS production.

Keywords: Neutrophils, CD13, Phagocytosis

Participation of neutral sphingomyelinases to *in vitro* virulence of *Entamoeba histolytica*

Fátima Ramírez-Montiel*, Ángeles Rangel-Serrano*, Luz Urquieta-Ramírez, Sairy Andrade-Guillén, Itzel Páramo-Pérez, Ruth Reyes-Cortes, Bernardo Franco, Claudia Leticia Mendoza-Macías, Fernando Anaya-Velázquez, Felipe Padilla-Vaca*.

Departamento de Biología y Departamento de Farmacia. División de Ciencias Naturales y Exactas. Universidad de Guanajuato

*Corresponding authors: fb.ramirezmontiel@ugto.mx , arangel@ugto.mx, padillaf@ugto.mx. Noria Alta s/n, Guanajuato, Gto 36050, México.

Amoebiasis is a worldwide health problem caused by the pathogen *Entamoeba histolytica*. Several virulence factors have been implicated in host invasion, immune evasion, and tissue damage. There are still new factors that remain to be elucidated and characterized. In this work, we obtained amoebic transfectants overexpressing three of the neutral sphingomyelinase enzymes encoded in the *E. histolytica* genome. The *EhnSM3* overexpression induced an increase in hemolytic and cytotoxic activities, besides an increase in gene expression of amoebapore A, B, and C. Meanwhile the *EhnSM1* and *EhnSM2* overexpression caused an increase in cytopathic activity. In all the neutral sphingomyelinases overexpressing strains, the gene expression levels for cysteine proteinase 5, adhesin 112 and, heavy and light Gal/GalNAc lectin subunits were not affected. We propose that the increase of cytotoxic and lytic effect of *EhnSM3* overexpressed strain is the sum of the effect of EhnSM3 plus amoebapores, in a process cell contact-dependent or as mediator by inducing the gene expression of amoebapores enabling a link between EhnSM3 with the virulence phenotype in *E. histolytica*. Our results suggest a differential role for neutral sphingomyelinases in *E. histolytica* virulence.

Cloning and expression of Cathepsin-B from *Fasciola hepatica* in *Escherichia coli*.

[1]Miguel Ángel Ramírez Valdivia, [2]José Alberto Cano Buendía and [1]Sandra Morales Arrieta.
[1]Universidad Politécnica del Estado de Morelos, Boulevard Cuauhnáhuac No. 566 Col Lomas del Texcal Jiutepec Morelos.
[2]Facultad de Medicina Veterinaria y Zootecnia-UNAM. Mexico.
rvmo150788@upemor.edu.mx – 7771956313

INTRODUCTION Fasciolosis is caused by *Fasciola hepatica*, found mainly in ruminants; such as, sheeps and calf.
This parasite infects snails of the genus *Lymnaea spp*; which is an intermediate host that is internalized into definitive hosts. These snails are accidentally consumed by animals. At the host's intestine, the parasite secretes Cathepsin-B (peptidase of the C1 family), which helps in the infection process giving access to the liver. Unfortunately, immune response is slow and by the time there is an antibody production, the animal will already be infected. Triclabendazole is the only effective drug against early stages of the parasite; however, resistance to it has been extensively reported [1].
Other control measures such as, vaccination should be developed for sustainable control of this disease [1]. The production of recombinant proteins in bacteria and its use as vaccines has promising advantages like: capable of triggering an effective and lasting immune response, [2] fastest growth, easily reachable high cellular density, easy genetic manipulation, cheapest method to produce high levels of protein, and facilitates scaling. The goal of this work is the evaluation of different *E. coli* strains in Cathepsin-B expression and it use as a candidate vaccine into avoid fasciolosis.
METHODOLOGY Cathepsin-B sequence was optimized for *E. coli* expression system using the protocol described by Borovkov [3]; NcoI and ApaI restriction sites were added at the start and the end of the sequence respectively and generated sequence gene was builded using overlapping annealing protocols, and cloned into a cloning vector.
Cathepsin-B optimized sequence was sub-cloned into an expression plasmid (pBAD/MycHis-A, ThermoFisher, V440-01) and used in bacteria transformation using molecular biology protocols. Plasmid was purified using a plasmid purification kit (GeneJET Plasmid Miniprep Kit, ThermoFisher, #K0502) and sent for for sequence confirmation.
Plasmid was amplified and used in transformation of BL21, Top10 and DH5α *E. coli* strains.
RESULTS Cathepsin-B gene was optimal cloned into a prokaryotic expression vector. The translated optimized sequence of Cathepsin-B showed a 100% identity with Cathepsin-B, and the nucleotide sequence showed 19 optimized codons. The obtained *in silico* modelling using Swiss model, revealed that Cathepsin-B sequence conserves its third structure, the enzyme have 376 amino acids, a molecular weight of 42.2 kDa, and a theoretical IEP = 5.66.
FUTURE WORK The expression levels will be evaluated and the enzyme will be produced in high levels, allowing the search of new alternatives to avoid fasciolosis.
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Exploration of innate immune responses of Northern elephant seal pups throughout their fasting period

Nami Morales-Duran, Cecilia Barragán-Vargas, Martin Stoffel, Karina Acevedo-Whitehouse

Laboratorio de Genética Molecular y Ecología Evolutiva, Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Carretera a Chichimequillas S/N, Ejido Bolaños, 76140 Santiago de Querétaro, Qro., (442)1921200 ext. 65334, karina.acevedo.whitehouse@uaq.mx

The function of the immune system is dynamic and depends on many factors that determine the efficiency of its response. This means that responses may be compromised when resources are limited, as the immune system will compete with other costly physiological functions, such as growth and reproduction. Competition for resources implies a reallocation of resources between different physiological functions. In natural populations, the distribution of resources will depend on the environmental context in which the individuals are found. Several studies have been carried out that try to understand the factors that affect the immunological responses in natural populations, as well as the costs caused by the host parasite interaction. However, little is known to be able to generalize about the exchanges in different ecological, ontogenetic and phylogenetic contexts. Studies conducted in birds have shown that growth and reproduction are comparable in terms of energetic cost with the activation of the immune system. Thus, immunity is an important life history character. Similar to other phocid species, the northern elephant seal (*Mirounga angustirostris*) has a very high selective pressure at birth, because it has to grow rapidly during nursing, which lasts only 28 days, and weaning is abrupt. Post weaning, pups remain up to 65 days on land, where they fast until ready to go to sea. We were interested in examining whether the *in vivo* response to a mitogenic challenge with phytohemaglutinin (PHA) and the *ex vivo* response to *E. coli* of newly-weaned Northern Elephant seal pips was dependent on their body size, as a proxy of available resources, and whether body size at day 1 of fasting predicted the magnitude of the response at the end (day 36) of their fasting period. We found that the magnitude of the response to PHA varied significantly between day 1 and day 36, being lower at the end of the fasting period than on the first day of fasting (Kruskal-Wallis, Chi^2=13.922, df=1, p=0.00029), similar to inhibition of *E. coli* (Kruskal-Wallis, Chi^2=12.65, df=1, p=0.0004).Furthermore, the magnitude of the response of PHA was influenced by umbilical girth, regardless of the period of fasting (GLM, $F_{1,32}$=4.539, R^2= 0.124, p=0.0409) implying that as maternally-derived resources decrease after weaning, pups become less able to respond to an immune challenge. This is the first study to examine innate immune effectors of a phocid species in the context of early life history.

CHARACTERIZATION OF A MUTANT OF THE ENZYME TRANS-SIALIDASE FROM *Trypanosoma cruzi* (TcTS) AND ITS POTENTIAL FOR A VACCINE DEVELOPMENT AGAINST CHAGAS DISEASE

Carlos Miguel Barajas Artega, Julio Cesar Villagómez Castro, Sabine L. Flitsch, Juana Elizabeth Reyes Martínez*
Departamento de Biología, División de Ciencias Naturales y Exactas, Campus Guanajuato, Universidad de Guanajuato, Noria Alta S/N, Guanajuato, Gto. C.P. 36050. Tel: (+52) 473-7320006 Ext: 8158, e-mail: juana.reyes@ugto.mx

Abstract

Chagas disease or American trypanosomiasis is an infection caused by the parasite *Trypanosoma cruzi*, this condition is characterized by presenting in its acute phase common symptoms such as fever, muscle pain and fatigue, and in its chronic phase generates affections to the heart and nervous system. Trypanosomiasis is a zoonosis transmitted to humans through the Triatomine bug, endemic to subtropical areas mainly Latin America, which also can be found in some regions of the United States and Europe. Currently the main treatment has an antiparasitic approach focused on antiparasitic drugs administration in acute phase (benznidazole and nifurtimox) which high toxicity has been observed in longer period's administration; up to date there is no vaccine against the disease.

T. cruzi is characterized by evading the immune system by adding sialic acid on its surface; however, *T. cruzi* is unable to synthesize this sugar *de novo*. Sialic acid is obtained from the host by the action of an enzyme called Trans-Sialidase (TcTS). Since the human lacks of proteins similar to this, it makes this enzyme a potential target for drugs and vaccines design. Is known, that the presence of specific antibodies to this enzyme correlate with lower parasitic load in animal models.

Previously, heterologous expression of this enzyme was done by introducing the catalytic site codifying sequence into pTrcHis expression vector successfully. In our group a colorimetric method to easily determine the enzyme activity was set and an inactive mutant was identified.

In this research work, we characterize the mutant by isolation the plasmidic DNA to perform PCR reactions for sequencing the ORF in order to determine specific mutations causing its inactivity. The mutant enzyme was heterologously expressed in *E. coli* and purified by Ni-NTA columns, once quantified the enzyme by Bicinchoninic Acid Assay, an immunization scheme was established in rabbits. The antibodies produced with will be isolated to characterize its functionality inhibiting the activity of wild type enzyme. Finally it is intended to determine the parasitic load in the presence of mutant anti-TcTS antibodies on "*in vitro*" infection.

The calcium homeostasis of the Golgi apparatus could be regulated by an SPCA in *Entamoeba histolytica*

Mario A. Rodríguez, Martha I. Valle-Solis, Mario. Hernandes-Alejandro, Ana H. Figueroa-Gutiérrez, Jesús Carlos Ruvalcaba Ledezma, David López-Romero and Andrés Salas-Casas*.

*Andrés Salas-Casas. Instituto de Ciencias de la Salud. Área Académica de Gerontología. Universidad Autónoma del Estado de Hidalgo. Ex Hacienda la Concepción, s/n; Carretera Actopan-Tilcuautla, San Agustín Tlaxiaca, Hidalgo, México. C.P. 42060. Tel (771) 1327538 ext. 4314. E-mail: andres_salas15@yahoo.com.mx

Abstract

There are three subtypes of calcium pumps that are phylogenetically distinct and marked according to their subcellular localizations: namely, the plasma membrane (Plasma Membrane Ca^{2+}-ATPase or PMCA), endoplasmic reticulum (Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase or SERCA), and Golgi/ Golgi-derived vesicles (Secretory Pathway Ca^{2+}-ATPase or SPCA). In this work we study a possible ATPase type SPCA of *Entamoeba histolytica*.

SPCA resides in Golgi apparatus (GA) compartments and post-Golgi vesicles and it is the main system of Ca^{2+} recovery in this organelle after cellular activation. Furthermore, this pump plays an important role in the correct regulation of protein traffic and secretion. Unlike to PMCA and SERCA, SPCA also transports manganese ions with high affinity and contrasting with SERCA, it does not exchange H+ in the process of transport. SPCA has a single binding site for Ca^{2+} and another for Mn2+ and the presence of these two binding sites possibly owes itself to the greater stability of the E1 over the E2 conformation.

The Golgi apparatus has an important role in secretory pathways also is crucial in controlling cell Ca^{2+}. It has important functions such as: post-translational modification, transit of secreted, function in sorting vacuolar, and lysosomal proteins, many of these membrane-bound compartments also serve as intracellular Ca^{2+} reservoirs. Recently it has been demonstrated with the help of various technologies that GA can store up to 5% of the total Ca^{2+}.

In conclusion, we identify a putative SPCA in *E. histolytica*. This assumption is based on that it contains all characteristics motifs of the SPCA family and is located in the membrane of vacuoles stained with NBD C6-ceramide. However, it is necessary to conduct further pharmacological and electrophysiological experiments to confirm this hypothesis. In addition, the knockdown strategy will be useful to explore the role of this Ca^{2+}-ATPase in calcium/manganese homeostasis and in signaling pathways triggered by Ca^{2+} in *E. histolytica*.

Suramin Evokes Two Effects on Human P2X Receptors of Macrophages

Eydie Mariela Vargas-Martínez, Rosa Espinosa-Luna, Luis Manuel Montaño-Ramírez, Carlos Barajas-López.
Instituto Potosino de Investigación Científica y Tecnológica, A.C.
Camino a la Presa San José 2055. Col. Lomas 4a. Sección. San Luis Potosí, SLP. CP: 78216. Tel: +52(444)834-2000 ext. 2033. Correo: cbarajas@ipicyt.edu.mx

P2X purinoceptors are ligand-gated ion channels stimulated by adenosine 5-triphosphate (ATP), they are found in all tissues where are play diverse physiological and physiopathological functions. In human macrophages the presence of P2X1 receptors, P2X4 and P2X7 has been reported, but the interaction to form functional channels is unknown. In addition, nonselective antagonists, such as suramin and PPADS have been used to characterize P2X receptors in heterologous expression. Therefore, the aim here was to determine if native P2X receptors from human macrophages are mainly mediate for P2X sensitive to suramin using the whole cell configuration of the Patch Clamp technique. Peripheral blood monocytes were obtained from healthy volunteers and cultured for a period longer than 7 days in the presence of GM-CSF to generate macrophages, these primary cultures were used in all experiments. The recording camera was constantly superfused with external solution. The pipettes had a resistance between 10-15 MΩ. The exchanging of solutions and drugs was made using eight-tube device. The membrane potential was set at -40 mV. All the experiments were performed at room temperature (~23 ° C). Records obtained from human macrophages beings incoming currents of rapid desensitization type P2X1 by the application of five seconds of ATP 10 μM, nonetheless, the application of suramin (0.1, 0.3, 10 or 30 μM) induced a dual effect that was concentration dependent, inhibition, and potentiation. On the other hand, with high levels of ATP (1-5 mM) currents were observed with two phases similar to those induced, in recombinant P2X1 receptors and non-desensitizing currents were also observed as reported in the recombinant P2X7 or P2X1del receptors, these responses show inhibition and potentiation after suramin application, 100 and 30 μM respectively. On the other hand, non-desensitizing response was permeable to large cations such as NMDG+. So far, our results with suramin differ from those reported on the human homomeric receptors (P2X1 IC_{50}≈ 1 μM while P2X7 and P2X4 IC_{50} > 100 μM). However, when the native receptors are exposed to this antagonist, the kinetics are not identical to the expected for homomeric or some heteromeric channels, perhaps as a result of receptor heterogeneity and distribution, which could result in various stoichiometries with different kinetics and pharmacological properties. kinetics. More experiments in heterologous expression are required to affirm these pharmacological behaviors.

Effect of three different organic compounds on the cytotoxicity of *Trichomonas vaginalis* toward HeLa cells.

Laura Isabel Vázquez-Carrillo[1] , Yussel Fernando Pérez Navarro[1], Gumersindo Mirón López[2], Julio César Torres Romero[2], Mauricio Castañón Arreola[1], María Elizbeth Alvarez-Sánchez[1*]
[1]Posgrado en Ciencias Genómicas, Universidad Autónoma de la Ciudad de México (UACM). San Lorenzo 290, Col. Del Valle, Ciudad de México..
[2]Facultad de Química, Universida Autónoma de Yucatán.
*corresponding author. Tel.: +52 5554886661x15306;.
mail: maria.alvarez@uacm.edu.mx; elizbeth2@gmail.com.

Trichomonas vaginalis is the causative agent of trichomoniasis,sexually transmitted disease. This parasite infects main to the women, whereas men are asymptomatic carriers. Metronidazole is the treatment of choice for trichomoniasis, however recently, crude extracts from medicinal plants had been tested against *T. vaginalis*. *T. vaginalis* has many proteinases involved in the cytotoxicity of the parasite toward the target cell,, such as the cysteine proteinases, TvCP65, TvCP39, and metalloproteinases as TvMP50. The aim of this work was to evaluate the effect of three different organic compounds (A7.11.11, A7.11.9 y A7.4.5+SPE-2) on the cytotoxicity of *T. vaginalis* toward HeLa cells. First, we determined the parasite viability in the organic compounds through a growth curve at different times, then we analyzed the proteinase activity of TvCP65, TvCP39, and TvMP50 on substrate-SDS-PAGE, the transcript levels of *tvcp65*, *tvcp39* and *mp50* by RT-PCR and the cytotoxicity of parasites (treated with the organic compounds, metronidazole or without treatment) toward the HeLa cells. We found that the parasites treated with the A7.11.9 y A7.4.5+SPE-2 compounds had the transcript levels *tvcp65*, *tvcp39* and *mp50* decreased in comparison with the parasites treated with metronidazole or the control parasites. These organic compounds downregulate the cytotoxicity and the proteolytic activity of TvCP65, TvCP39 and TvMP50 . We suggested that these organic compounds could be used in the future as a new chemotherapeutic for the trichomoniasis treatment

Gut Mucopolysaccharide Levels in Mice Treated with Bovine Lactoferrin

1Vega-Bautista Alan, 1Melendez-Avalos Araceli, 2Sánchez-García Quetzalcóatl, 3Godínez-Victoria Marycarmen, 3Campos-Rodríguez Rafael,1Drago-Serrano Maria Elisa (dragome@yahoo.com)
1 Depto. Sistemas Biológicos, UAM-Xochimilco, 2Depto. Bacteriología, Escuela Nacional de Ciencias Biológicas, IPN, 3 Secc. Estudios de Posgrado e Investigacion, Escuela Superior de Medicina, IPN. Working place: Lab Microbiología Molecular, UAMX, Calzada del Hueso No. 1100 CDMX 04960, CDMX, Tel +5255 54837000 ext 3624/3652

Introduction. Mucopolysaccharides (Mucopol) are the main components of gut *mucus* that display an anti-inflammatory role by decreasing the direct contact of epithelial surface with microbiota. Small intestine is covered by a single loose (unattached) *mucus* layer whereas the colon is covered by an inner *mucus* layer firmly attached to the epithelial surface, and upon it an outer loose *mucus* layer. Bovine lactoferrin (bLf) is a multifunctional glycoprotein whose modulatory effects on gut Mucopol are unknown **Aim**. To assess the influence of bLf on Mucopol content in terms of intestinal regionalization **Methods**. Groups (n=4) of 8 week-old BALB/c mice were treated 4 days with either 0.5 % or 5% bLf (female) as well as 4 or 7 days with 0.5 % bLf (male). Water (control group) or bLf were *ad libitum* administered within drinking bottles. Mice were euthanized with isoflurane. The intestinal tract was collected and flushed with isotonic saline solution to remove feces before dissecting the proximal region (next to pylorus) and distal colon (next to rectum). Strips of 1 cm in length of each region were weighed and stained with alcian blue solution and then the blue complexes were extracted with 30 % sodium docusate in 70% ethanol. Mucopol levels in µg/g were quantified with a standard curve by plotting the different concentrations of chondroitin 4-sulfate *versus* the absorbance values at λ=620 nm. Two experiments were done, and data were expressed as mean and standard deviation and analyzed by one-way ANOVA. Significant differences were regarded at $p<0.05$ **Results**. In female mice, 5 % bLf-treated group had lower Mucopol levels in both intestinal segments in regard the control and the 0.5% bLf-treated groups. No differences were found between these latter groups. In male mice, Mucopol levels in 7-days bLf-treated group were lower than 4-days bLf-treated and control groups at proximal region as well lower than 4-days bLf-treated group in the colon; no differences were found between control and 4-days bLf treated groups **Discussion**. Data indicated that 0.5 % bLf treatment during for 4 days unaltered the normal Mucopol content in the proximal region and colon from female and male mice. In contrast, data suggested a potential inflammatory effect by decreasing the Mucopol content by the 5% bLf treatment for 4 days in female mice and the 0.5% bLf treatment for 7-days in male mice **Perspectives**. To optimize the bLf treatment to explore the modulatory effects on gut homeostasis.

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Production, characterization and epitope mapping of a novel anti-N-truncated/pyroglutamate-modified AβN3(pE) peptide

David Vladimir Venegas Jerónimo, Gonzalo Acero, Goar Gevorkian
Instituto de Investigaciones Biomédicas, UNAM, Ciudad Universitaria, C.P. 04510, CDMX. Tel: 5556223151. gokar@unam.mx

The accumulation of fibrillar and oligomeric forms of amyloid-beta (Aβ) peptide in the brain has been hypothesized to play a central role in the neuropathology of Alzheimer's Disease (AD). The main Aβ variants detected in the human brain are Aβ1-40 and Aβ1-42, however a significant proportion of AD brain Aβ consists also of N-terminal truncated/modified species. These shortened Aβ forms are significantly more resistant to degradation, aggregate more rapidly *in vitro,* exhibit increased toxicity and progressively accumulate in the brain of Familial Alzheimer's disease (FAD) and Down syndrome patients as well as in the brain of sporadic AD patients at the earliest stages of AD even before the appearance of clinical symptoms. Thus, the N-terminally truncated/modified Aβ peptides represent highly desirable and abundant therapeutic targets and diagnostic markers.

In the present study we have focused on N-truncated/modified AβN3(pE), a major component of the amyloid deposits in the AD brain. Using spleen cells from AβN3(pE)-immunized mice, we produced a monoclonal antibody (3B8) that specifically binds to AβN3(pE) in enzyme-linked immunosorbent assay (ELISA) and Western Blot. In addition, 3B8 antibody binds to amyloid aggregates present in AD brain samples. Also, we performed epitope mapping using phage displayed peptide library and demonstrated that 3B8 recognizes a conformational epitope.

We believe our results are potentially important for developing new immunotherapeutic compounds specifically targeting AβN3(pE) aggregates, since the commonly used immunogens in the majority of vaccine strategies for AD have been shown to induce antibodies that recognized the amino-terminal part (EFRH epitope) of the full length Aβ, which is absent in N-amino truncated peptides. Also, the diagnostic potential of 3B8 should be explored.

Exposure to an Enriched Environment Attenuates Mouse Experimental Colitis

Tomás Villaseñor1, Porfirio Nava2, Carolina I. Serrano-García2, Victor Osio Becerro, Leonor Pérez-Martínez1 and Gustavo Pedraza-Alva1*
1 Laboratorio de Neuroinmunobiología, Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología, Universidad Nacional Autónoma de México. Cuernavaca, Morelos, CP 62210. México.
2 Departamento de Fisiología, Biofísica y Neurociencias. Centro de Investigación y de Estudios Avanzados (CINVESTAV), Instituto Politécnico Nacional. C. P 07360. Ciudad de México
*gustavo@ibt.unam.mx

It is known that somatosensorial stimulation through exposure to enriched environments enhances central nervous system functions and improves homeostasis (Nithianantharajah & Hannan, 2006; Reichmann, Painsipp, & Holzer, 2013). In addition to the central nervous system, the functions of the immune system are also modulated by exposure to an enriched environment, improving phagocytosis, chemotaxis and attenuating the inflammatory response induced by lipopolysaccharides (Arranz et al., 2010). In this work, we show that exposure to an enriched environment attenuates inflammation in the colon. After dextran sodium sulfate (DSS) or Trinitrobenzenesulfonic acid (TNBS) treatment, animals exposed to an enriched environment showed reduced weight loss, reduced colon shortening and disease activity score than animals housed in a normal environment. Accordingly, the colon of animals exposed to an enriched environment showed reduced epithelial damage, less immune cellular infiltrate, reduced myeloperoxidase activity and secreted lower TNF, IL-6 and IL-1b levels than animals housed in a normal environment. In contrast, colon explants from mice exposed to an enriched environment produce higher levels of the anti-inflammatory cytokine IL-10, in response to DSS or TNBS, than the colon explants from animals housed in standard conditions. In agreement with the fact that exposure to an enriched environment attenuates inflammation and reduces epithelial damage in the colon, we found that animals exposed to the enrich environment had low levels of LPS and other pathogen associated molecular patterns in circulation, compared with animals housed in normal conditions. Together our results show that brain stimulation by exposure to an enriched environment attenuates inflammation in the gut mucosa, resulting in improved epithelial barrier functions.

Keywords: enriched environment, BDNF, Inflammation, Colitis, somatosensorial stimulation

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Study of calcium signaling generated by activation of a photosensitizing substance and its propagation in prostate cancer cells

María Coral Aguilar Santos, Luis Alfredo Landa Montes, Laura Guadalupe Hernández Aragón, Fabián Galindo Ramírez*.
*Instituto de Fisiología. Benemérita Universidad Autónoma de Puebla. 14 sur 6301 Jardines de San Manuel. Puebla, Puebla C.P. 72570 Tel. (222) 2295500 Ext. 7324. Email: fabian.galindo@correo.buap.mx

Cancer research has worldwide impact; at least one in five individuals will develop a neoplasm in its life, prostate cancer in Mexico has the higher incidence and mortality rate in the male population. There are several treatments with curative purposes, among them the photodynamic therapy, it uses a photosensitizing substance, a specific light stimulus in the affected area and molecular oxygen which leads to the production of reactive oxygen species and finally cell death by cytotoxicity.
It is also known that during photostimulation calcium waves are generated, these could facilitate tumor damage and transmit it even to cells that are not directly irradiated (bystander effect). This process can be mediated through gap junctions and hemichannels formed by connexins.
In order to study the calcium signaling after photostimulation and the importance of connexins in signal propagation photodynamic therapy was applied to the prostate cancer cell line PC-3 with joint administration of non-specific blockers of gap junctions and hemichannels.
The results indicate that during photostimulation a rise in the intracellular calcium levels is generated which is capable of propagating to cells that are not directly irradiated. The amplitude of the produced signals is dependent on the distance; these two variables have an inversely proportional relationship.
The administration of non-specific blockers of gap junctions and hemichannels reduced the amplitude of the calcium signaling in a distance-dependent manner, which suggests the participation of hemichannels in the bystander effect. In addition, immunofluorescence technique corroborated the membrane presence of Cx43 and perinuclear location of Cx26, concluding that the effects made by the blockers act mainly in structures formed by Cx43. However, other less studied types of connexins may have a roll in the calcium signals in PC-3 cells.

Expression of the transcription factors HIF1a, HIF2a and HIF3a in pulmonary fibroblasts exposed to hypoxia.

Arnoldo Aquino-Gálvez*, Yair Romero López, Georgina González-Ávila, Javier Delgado Tello, Hector A. Maldonado Martinez, Víctor Hugo Olivera Rodriguez, Laura L. Jimenez Sanchez, José G. Cisneros Lira, Manuel Castillejos-López, Luz María Torres-Espíndola, Marco Checa Caratachea, Joaquín Zúñiga Ramos.
National Institute of Respiratory Diseases "Ismael Cosío Villegas," Calzada de Tlalpan 4502, Tlalpan CDMX, CP 14 080. Tel 54871700 Ext 5287. e-mail. araquiga@yahoo.com.mx

Keywords: Hypoxia, Pulmonary fibrosis, Smooth muscle alpha-actin

ABSTRACT

Background: Idiopathic pulmonary fibrosis (IPF) is a progressive, irreversible and usually fatal disease of unknown etiology. Hypoxia has been described as a determinant factor in the development and progression. However, the role of distinct members of this pathway is still unclear.

The aim of this work was to evaluate the transcription factors HIF1a, HIF2a, HIF3a and smooth muscle alpha-actin (ACTA) gene expression and basal protein levels at 48 hours of hypoxia exposure of fibroblasts from healthy and fibrotic lungs.

Methods: By immunohistochemistry, we observed the expression of proteins in tissue of lungs of IPF patients. Healthy and fibrotic fibroblasts were cultured under conditions of hypoxia (1% O_2). qPCR and Western Blot were used to determine the expression of HIF1a, HIF2a, HIF3a, ACTA-2 in lung fibroblasts isolated from healthy donor and IPF patients after hypoxia.

Results: Hypoxia signaling pathway is active in lungs derived from IPF patients by the abundance of two alpha subunits (1 and 2). Likewise, IPF derived fibroblasts showed a significant increase of these proteins ($p < 0.05$). In sharp contrast, HIF3a has a reduced expression in IPF (lungs and derived fibroblasts) which is correlated to the increased expression of fibrotic marker as αSMA.

Conclusion: Hypoxia may play a role in the development of fibrosis probably involving overexpression of pro-fibrotic marker such as ACTA.

Participation of the STIM1 / Orai1 complex in cell proliferation and migration, induced by epidermal growth factor (EGF) in the MDA-MB 231 cell line.

Avalos-López Juan Manuel, López-Cuevas Julieta, Monjaraz-Guzmán Eduardo
Laboratorio de Neuroendocrinología. Instituto de Fisiología-Benemerita Universidad Autonoma de Puebla. Calle 14 sur 6301. Col. San Manuel, Puebla. C.P. 72570 Tel. 2 29 55 00 ext. 7311 Email: big.pim@hotmail.com

Triple-negative breast cancer represents 15% to 20% of all cases of breast cancer, presents the most aggressive phenotype and the worst prognosis of survival. These tumor cells are characterized by being negative for the expression of ER α, PR and HER2, so there is no efficient pharmacological treatment so far. These cells overexpress the receptor to EGF, whose activation exacerbates its metastatic potential, and the molecular mechanisms involved are still unknown. Various evidences show the participation of calcium signaling in tumor development and progression. The entry of calcium through the SOCE pathway (Orai1 / STIM1), represents one of the key elements that favors the proliferative and migratory capacity in retinoblastoma cells. In the present project, we explored whether the effect induced by EGF on the proliferative and migratory capacity of triple negative breast cancer cells is associated with changes in the functional expression levels of the Orai1 / STIM1 complex.

METHODS

The MDA-MB 231 cell line was employed as an experimental model, which was maintained in a culture medium in the presence of EGF for 96 hours. By endpoint RT-PCR assays, the expression levels of the mRNA encoding Orai1 and STIM1 were detected. In order to evaluate the functional capacity of the SOCE pathway, microspectrofluorometry assays were performed, detecting cytosolic free calcium levels in MDA-MB 231 cells pretreated with EGF. The proliferative capacity was determined by BrdU incorporation assays, and to evaluate the migratory capacity, transwell chamber assays.

RESULTS

The tonic activation of the receptor to EGF regulates the level of expression of the mRNA that codes for Orai1 and STIM1, as well as the levels of free cytosolic calcium, the proliferative and migratory capacity of the MDA-MB 231 cells, which will help to understand a little more about the molecular mechanisms involved in tumor progression and development, and will allow, in the medium and long term, to propose new pharmacological targets for the efficient treatment of triple negative breast cancer.

Salivary MMP-2 activity in Type 2 Diabetes Mellitus patients with Periodontitis

Brenda Ayerdi Nájera, Arvey Tacuba Saavedra, Napoleón Navarro tito, Gladys León Dorantes, Nubia Blanco García, Juan Antonio Arreguin-Cano*
Unidad de Innovación Clínica y Epidemiológica del Estado de Guerrero. Av. Juan R. Escudero No. 158 Col. C.D. Renacimiento 39715 Acapulco de Juárez Guerrero México, Cel. 55 48 71 89 42
arreguin90@hotmail.com

Matrix metalloproteinases (**MMPs**) are important molecules in physiological processes such as reproduction, embryonic development and pathologic tissue remodeling. Additionally, MMPs can process various nonmatrix bioactive substrates such as proinflammatory and anti-inflammatory cytokines, chemokines, growth factors, serpins, serum components, complement components, and cell signaling molecules, therefore modifying immune responses. MMP-2, a 72-kDa type IV collagenase, is a member of this MMP family that plays a significant function in several physiological or pathological processes. Thus, their overexpression is often associated with different diseases.

In the oral cavity MMP-2 is synthesized by fibroblast, endothelial cells and osteoblasts. The main source of the MMP-2 found in saliva is the polymorphonuclear leukocyte. Its activity can be regulated by tissue inhibitors of Matrix metalloproteinases (**TIMPs**), particularly TIMP-1, that is an endogenous inhibitor of MMP-2 produced by periodontal cells, macrophages, and monocytes. In periodontal tissue inflammation, MMPs augmented expression leads to increased MMP levels in biological fluids such as saliva and gingival crevicular fluid. In addition, clinical studies suggest that high circulating MMP-2 levels may correlate with the severity of microangiopathic complications in advanced stages of Type 2 Diabetes Mellitus (**DMT2**), but its significance in early stages is still unknown. Likewise, uncontrolled DMT2 increases the risk of developing periodontitis.

Objective: To compare MMP-2 activity and TIMP-1 salivary levels in patients with Type 2 Diabetes Mellitus with and without periodontitis.

Methodology: The present study received approval from the Ethics Committee of Guerrero's Secretary of Health. After signed informed consent DMT2 patients were included. Measurements: periodontal disease severity according to the American Academy of Periodontology criteria in three groups: mild, moderate and severe; percentage of glycosylated haemoglobin, time of DMT2 onset, and saliva samples. These samples were analyzed by zymography to detect gelatinolytic activity of MMP-2 and by Western-blot to determine TIMP-1 levels. In both essays, the integrated optical density (imageJ®) was used to obtain the results. **Analysis.** Results were analyzed be Student's T Test.

Results: Ninety-seven DMT2 patients were included. 67 (69.79%) had periodontitis and 30 (31.25%) not. Severity of those with periodontitis was found in 20 mild (29.85%), in 14 moderate (20.89%), and in 33 severe (49.26%). MMP-2 activity and TIMP-1levels were significantly increased in patients with severe periodontitis compared with healthy individuals. Severity of periodontitis significantly increased with the years of onset of DMT2. On the other hand, in those patients with higher percentages of glycated haemoglobin, the activity of MMP-2 and levels of TIMP-1 decreased. Also, the increase in the percentage of HbA1c significantly correlated with an increase the periodontal disease severity

Conclusions: In this study we could observe the positive correlation of time of onset of DMT2 with the severity of periodontal disease. Also, uncontrolled DMT2 defined by higher percentages of HbA1c negatively correlated to MMP-2 activity and TIMP-1 levels. These results suggest that periodontal remodelling capacity of MMP-2/TIMP-1 is affected proportionally to the increase of the percentage of HbA1c and the development of periodontitis in these patients.

Changes of urokinase-type plasminogen activator in breast cancer cells in presence of nicotinamide.

María Guadalupe Martínez Hernández[1], Araceli Rubí Viedma Rodríguez[1], Margarita Díaz Flores[2], Luis Arturo Baiza Gutman[1].
[1]Morphology and Function Unity, Faculty of Professional Studies Iztacala, UNAM. [2]Biochemistry Investigation Unit, Specialities Hospital, CMN Siglo XXI, IMSS.Av. De los Barrios No. 1 C.P. 54090 Tel: 56231261 hermargu@unam.mx labaiza@unam.mx

Cancer invasion in tissue entails the activity of extracellular proteases, such as urokinase-plasminogen (PLAU) and matrix metalloproteinases (MMPs). PLAU plays an important role in the malignancy and progression of the tumor. It is secreted to the extracellular medium and its expression has been related to aggressiveness in tumors. Reactive oxygen species (ROS) can regulate the activity of transcription factors like nuclear factor kβ (NF-kβ). The promotor region of PLAU contains a union element to NF-kβ, which is sensible to redox changes. Antioxidants, in this case nicotinamide, can reduce ROS regulating the expression, secretion and activity of PLAU associated with the invasive ability of cancer cells. Our objective was to evaluate the effects of nicotinamide on cellular viability, apoptosis and secretion of PLAU in the breast cancer cells MDA-MB231. The growth of cell population was evaluated through MTT trail, apoptosis was determined using double staining Annexin V-FITC/IP through flow cytometry. Secretion was evaluated with the help of gel zymography and the expression with Western blot. The data showed that depending on the dose of nicotinamide there was a decline in the growth of the cell population, the induction of apoptosis and a diminution in the secretion of PLAU. In conclusion the induction of cytotoxicity by nicotinamide in the breast cancer cells MDA-MB231 inhibits the secretion of PLAU and reduces the invasive activity of cancer cells. This is caused probably through the reduction of ROS.

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Identification of new mutations in *DYRK1B* associated with monogenic metabolic syndrome

Francisco Barajas-Olmos, Elvia Mendoza-Caamal, Humberto García-Ortiz, Federico Centeno-Cruz, Cecilia Contreras-Cubas, Adriana Reséndiz-Rodríguez, Isabel Cicerón-Arellano, Rigoberto Rosendo-Gutierrez, Angélica Martínez-Hernández, Elahe Mirzaeicheshmeh, Ian Ilizaliturri, and Lorena Orozco*
Instituto Nacional de Medicina Genómica, México, C.P. 14610, *lorozco@inmegen.gob.mx

Introduction. Recently, a new form of metabolic syndrome (MetS) with an autosomal dominant inheritance pattern has been reported. The causal mutations, Arg102Cys and His90Pro, are both located in the kinase domain of the *DYRK1B* gene, which plays an important role in adipogenesis by inhibiting the Sonic Hedgehog pathway and increasing the expression of C/*EBPa* and *PPARg* genes. Thus, the identification of new mutations within DYRK1B related to MetS are important to provide more insights of the role of this gene in this disease/pathology.

Objective: Identify mutations in *DYRK1B* causal of MetS in the Mexican population.

Material and methods: We searched for mutations within *DYRK1B* in data from the exome of 968 individuals (Human-All-Exon) with and without diagnosis of MetS, using the GATK, PolyPhen-2, and SIFT programs. Once the mutations were identified, all available relatives of the index patients were evaluated for medical history, blood chemistry and *DYRK1B* sequencing by Sanger, to determine the segregation of the mutations with the entity. To understand the effect of the mutations on the structure of the protein, in silico analysis was performed with NAMD.

Results: Here we report two new *DYRK1B* mutations, Lys68Gln and Arg252His, in two families with MetS. The multigenerational study showed the cosegregation of both mutations with MetS. Interestingly, in both families, the MetS is characterized by abdominal obesity (AO), hypertension and hyperglycemia rates. Moreover, in each family, one individual under 20 years old was found with the mutation and altered metabolic parameters. In the family with the Arg252His mutation, we found an adolescent of 11 years of age with AO, serum glucose of 105mg/dl, pre-hypertension, and hyperinsulinemia. For the second mutation, Lys68Gln, an 18-year-old individual with dyslipidemia, AO and blood pressure of 131/74 mmHg, with medical treatment and lifestyle intervention was found. The Root Mean-Square Deviation and Fluctuation documented that the structure of the protein derived from the Arg252His mutation presents a structure alteration in the kinase domain (370–390 aa). A deeper analysis showed that in the native structure the Arg252 forms hydrogen bonds with Pro554 and Ser552, while in the mutated structure, the His252 doesn't.

Conclusion: In this work, we report for the first time, 2 new mutations within *DYRK1B*, causing MetS with a dominant autosomal pattern of inheritance. With these two new cases, there are 4 families reported in the world with this syndrome.

ER-β activation regulates expression of VEGF-C / VEGFR-3 complex in triple negative breast cancer cells.

Zuleyma Basilio Lino, María Julieta López Cuevas, Eduardo Monjaraz Guzmán; Laboratorio de Neuroendocrinología, Instituto de Fisiología, Benemérita Universidad Autónoma de Puebla (BUAP). Avenida 14 Sur 6301, Col. San Manuel, CP 72570, Puebla, Pue. Tel. (222) 2 29 55 00 ext. 7311
Email: Zuleyma.Basilio@gmail.com

BACKGROUND

Breast cancer is the most common malignancy in women. Triple negative breast cancer represents 15 to 20% of all cases of breast cancer, being the most aggressive form with the worst prognosis of survival, causing more than half of all deaths caused by this cancer. These tumor cells are characterized by lack expression of ERα, PR and HER2, so there is no efficient pharmacological treatment so far. These tumor cells express ER-β and their functional relevance is unknown up to now. Several publications show the stimulatory participation of VEGF, specifically the C isoform, in the development and tumor progression. Therefore, the purpose of the present study is to investigate whether the activation of ERβ influences the expression levels of VEGF-C and its specific receptor VEGFR-3 in triple negative breast cancer cells, as well as its proliferative and migratory capacity.

METHODS

The MDA-MB 231 cell line was used as an experimental model, which was maintained in an estrogen-free culture medium. The cells were incubated in the presence of DPN, a specific agonist of ER-β. By end-point RT-PCR assays, the expression levels of the mRNA encoding VEGF-C and VEGFR-3 were detected. In order to evaluate the proliferative capacity, we carried out incorporation assay of BrdU, and to evaluate the migratory capacity, we carried out assay in transwell chambers.

RESULTS

Activation of ER-β by DPN regulates positively the level of expression of the mRNA encoding VEGF-C and VEGFR-3, as well as the proliferative and migratory capacity of MDA-MB 231 cells. This will help us to understand a little more about of the molecular mechanisms involved in the progression and tumor development, and will allow to propose new pharmacological targets for the efficient treatment of triple negative breast cancer.

Immunophenotypic characterization of GMP-grade mesenchymal stem cells derived from human adipose tissue

Mayra Y. Bazán-Álvarez, Lauro Gómez-Guerra, Nidia K. Moncada-Saucedo, Alejandro García-Ruiz, Vanessa Pérez-Silos, Susana G. Cárdenas-Ramos, Alberto Camacho, Erika Coronado-Cerda, Gabriel Mecott-Rivera, Lizeth Alicia Fuentes-Mera [1,2.]
[1]Laboratorio de Medicina Regenerativa. Departamento de Bioquímica de la Facultad de Medicina de la Universidad Autónoma de Nuevo León (UANL).
Madero y Dr. Aguirre Pequeño Col. Mitras Centro sin número. 64460, Monterrey, N.L., México.
2 +52 (81) 83294173 ext 2588; lizeth46@hotmail.com

Introduction: Vascular Stromal Fraction (SVF) is a heterogeneous mix of cells that includes adipose derived mesenchymal stem cells (ADSCs), among others, and because of this heterogeneityin the past years it has gained great significance in Regenerative Medicine.Stem cell therapy using SVF is a promising treatment modality forerectile dysfunction, since the mesenchymal stem cells can differentiate into several cell types, including smooth muscle, neurons, and of great relevance for the treatment of this condition,endothelial cells. Nevertheless, there is little information about the immunophenotyping and functional characterization of this fraction when it is isolated under the rigorous system that demands the clinical application, which is the use of GMP grade enzymatic systems.
The main goal of this study was to standardize the procedure for the isolation of the SVF from human adipose tissue using the GMP grade NB6 collagenase and subsequently, immunophenotyping the cells using flow cytometry approach.
Methodology: The adipose tissue sample derived from liposuction was washed with PBS and digested for 30 minutes at 37° C with the enzyme NB6 (GMP grade). A fraction of the SVF isolated was taken to cell culture and the rest was maintained for analysis by flow cytometry. The cells (freshly isolated and cultured) were labeled with monoclonal antibodies for the following surface markers: CD73, CD105, CD90, CD29 (mesenchymal lineage); CD117, CD38, CD45, CD34 (hematopoietic lineage) and CD24, CD326 (epithelial lineage) and analyzed in a BD Accuri ™ C6 Plus flow cytometer.
Results: We compared the population isolated with different concentrations of NB6 collagenase (0.2 vs. 0.1%), and we observed that a higher concentration of the NB6 enzyme determines the enrichment of a mesenchymal population with a positive expression for CD34. Immunophenotyping of the isolated SVF showed that >97% of the population co-expressed the mesenchymal surface markers CD73, CD105, and CD90. We also found a sub-population expressing CD34 marker (>79%), which is desirable in this approach of treatment. Regarding the epithelial markers, we found a low expression for CD24 and CD326 (2.7 and 1.4% respectively). The expanded cells from SVF retained the expression of all the mesenchymal markers after the first passage; however, the expression of hematopoietic and epithelial markers was negative.
Conclusion: Isolation of the SVF by digestion with the 0.2% NB6 enzyme allows the enrichment of a mesenchymal lineage population, being predominant and with special attention to the expression of the CD105 marker (related to vascular remodeling); also, the presence of a CD34 + population which is relevant since it is capable of efficiently delivering cells of the hematopoietic lineage.

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Long-term analysis of the electrocardiogram as a method of evaluation of the development of hypertrophy in conscious rats with spontaneous hypertension

Bocanegra Alfaro Ana Karina[1], De Jesús Luis, Gómez Villalobos María De Jesús

Institute of Physiology, BUAP, 14 sur 6301 Jardines de San Manuel, Puebla, 72570, México
E-mail: hanna_kari@hotmail.com, cell phone: (045) 8712555552

Cardiac hypertrophy (CH) is a compensatory mechanism secondary to chronic pressure overload, as observed in hypertension. Spontaneously hypertension rat (SHR) is a genetic model of hypertension that reproduces the pathology and cardiac hypertrophy. The aim of this work was to analysis the changes of the electrocardiogram (ECG) recording from one to eight months old in SHRs, to get information about the development of hypertrophy. Male wistar Kyoto (WK) and SH rats at one month old were used. Body weight (BW-g), systolic and diastolic blood pressure (SBP, DBP-mm Hg), heart rate (HR-beat/min) and electrocardiogram (ECG) bipolar derivation D1 (QRS morphology and R+S amplitude in mv) were measured and recorded each month during 1 to 8 months through non-invasive methods. At the end and under general anesthesia the hearts were removed and weighted. The ratio of heart/body weight was determined. Data are shown in mean ± SE. The results at one month old age were similar in body weight and SBP and DBP, in the experimental groups, however significantly differences in the ECG morphology and QRS amplitude were found. ECG in WK rats showed an ECG patter of a normal heart (Rs morphology, with amplitude of 308.35±9.3), whereas in SH rats we found two ECG patterns; one related to RS morphology and amplitude of 264.51±8.2, compatible with mild to moderate ventricular hypertrophy; and the second pattern with rS morphology and amplitude of 382.14±11.2 being compatible with severe heart hypertrophy. At two months of age there were significant differences between the two experimental groups, in the SBP (176.8 ± 14.01 and 154.2 ± 0.91), DBP (136.61 ± 26.49 and 104.2 ± 0.91) and HR, (344.1 ± 13.87 and 289.6 ± 17.77), respectively for SHR and WKR. P <0.001 between groups for each parameter. However the ECG pattern remains with minimal changes. During the next sixth months, there were minimal changes in the parameters monitored. At the end of the protocol and in accord with the heart weight, the ratio heart/body weight, the QRS morphology and R+S amplitude, hearts were classified as normal in WKR (1.18 ±0.04, 0.319±0.024, Rs morphology, and amplitude of 351±16). In SHR with mild to moderate cardiac hypertrophy the results were; 1.21±0.016, 0.391±0.012, with RS morphology, and amplitude of 251±16 and in severe cardiac hypertrophy were 1.42±0.055, 0.40±0.024, with rS pattern and 628±110, respectively. P<0.05, SHRs with severe cardiac hypertrophy against WKRs. The most important finding in this study, was that the ECG pattern compatible with cardiac hypertrophy was present at one month of age of the SH rats, before the hypertension is generated, and is sustained with minimal to moderate changes until eight months old age rats, suggesting that cardiac hypertrophy has a genetic origin and is not only developed as consequences of sustained hypertension.

Participation of the heat shock protein 27 in the regulation of the death of chondrocytes in the articular cartilage of an experimental model of osteoarthritis.

Moisés Cabrera-González (1), Cecilia González-Calixto (2), Raymundo Cruz (1), M. M. Miranda Sánchez (1), Juan B. Kouri Flores (1).
1.- Departamento de Infectómica y Patogénesis Molecular, CINVESTAV-IPN Av Instituto Politécnico Nacional 2508, La Laguna Ticoman, 07360 Ciudad de México, CDMX, e-mail: cabreragmoises@gmail.com. 2.- Facultad de Enfermería No.2, UAGro.

Introduction. Osteoarthritis (OA) is degenerative joint disease, it is a complex disease whose pathogenesis, changes the tissue homeostasis of articular cartilage and subchondral bone [1]. Chrondrocyte death, may result in the same outcome in view of the failure to appropriately maintain the structure of articular cartilage [2]. In various cell types, it has been documented that apoptosis is regulated by heat shock proteins (HSP) [3]. The HSP27, is of stress proteins that are well characterized in the role of resistance to apoptosis as it interacts directly with the components of apoptotic signaling pathways. Although the expression in the articular cartilage of HSP27 has been detected, its role in the regulation of programmed death of chondrocytes has not been determined [4]. **Objective.** Analyze the role of HSP27 in regulating the programmed cell death of chondrocytes will contribute to a better understanding of the pathogenesis of OA and will allow the establishment of potential therapeutic targets. **Materials and methods.** OA was induced in male Wistar rats by medial partial menisectomy (OA group) and exercise for 5, 10 and 20 days. As a control, a group with sham surgery (Sham) was used. The cryosections were prepared to perform indirect immunofluorescence, to detect anti-hsp27 and anti-pro-caspase 3. For the analysis of proteins were detected by western blot (WB) using specific anti-hsp27, and anti-pro-casp3 antibodies. **Results.** The results obtained from the WB showed an increase in the expression of HSP27 of up to 1.5 times to 20 days of induction of OA, compared to the control group. When we analyzed the interaction of Hsp27 and casp3, we observed an expression greater than 0.5 times at 10 days in the OA group when compared with the control group. **Discussion.** However, the increase in the expression of the Hsp27 protein in the groups with OA and the interaction with proapoptotic factors, the pathology continues to progress, which suggests that the anti-apoptotic function of this stress protein is minimized by all the catabolic processes that the chondrocyte has, like the expression of IL-1β and TNF-α, and that it has already been reported that you are cytokines that negatively regulate the expression of Hsp27 [4].

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Effect of the intranasal vaccine HB-ATV-8 on atherogenesis and non-alcoholic fatty liver disease in a rabbit model of atherosclerosis.

Sandra Calixto Tlacomulco, Roxana Gutiérrez Vidal, Jaime Mas Oliva. Instituto de Fisiología Celular, UNAM. Cto. Exterior,, Ciudad Universitaria, 04510 Ciudad de México. Tel. +52 55 (5255) 56225584 jmas@ifc.unam.mx

Cardiovascular diseases are a group of heart and blood vessels disorders, which represent theprincipal cause of death in the world. The underlying disease within the cardiovascular disorders is atherosclerosis, considered a systemic inflammatory process characterized by the accumulation of lipids and macrophages inside the intima of the arteries. The process of plaque formation is asymptomatic, but the rupture of the plaque or the endothelial erosion can induce the formation of thrombi, which in turn, can induce a myocardial infarction or an ischemic stroke. It has been proven that high levels of LDL cholesterol are directly related to the risk of the atherogenesis, while HDL cholesterol is known to prevent inflammation, oxidative stress and promotes cholesterol efflux to reduce the formation of injuries. CETP inhibition has become an antiatherogenic target due to patients with mutations on the CETP gene were identified as the molecular defect directly related to increased HDL-C levels. In addition, it has also been observed a substantial reduction of the LDL-C. Moreover, it has been found that mice which do not present CETP activity in plasma, have high levels of HDL-C and resistance to development of atherosclerosis induced by diet. In the present study the effect of vaccine HB-ATV-8 was evaluated upon lipid levels in serum, as well as, its effect in the formation of the atherosclerotic plaque and non-alcoholic fatty liver disease. Three groups were studied: a control group fed a chow diet; a group of rabbits fed a high fat diet supplemented with 1% of cholesterol; and a group fed the same high fat diet, but treated with a nasal application of 50µL of the HB-ATV-8 vaccine for 2 months. At the end of the experiment, rabbits were sacrificed and aorta and liver tissue were obtained and examined using optical microscopy, two-photon and second harmonic generation microscopy to determine atherosclerotic and hepatic lesions. It was found that the vaccine HB-ATV-8 is capable of stimulating the immune system of the rabbits during treatment, generating antibodies anti-CETP. HV-ATB-8 administration reduced atherosclerotic and hepatic lesions induced by the atherogenic diet. Taking into account the limited number of animals in each group no statistically significant differences were observed.
However, the administration of the HB-ATV-8 vaccine in general influences the decrease of plasma triglyceride levels (34%), total cholesterol (19%), and LDL-C (26%) in comparison with the high-fat diet group, while HDL-C levels increases 11% in contrast to the control group. These findings suggest that vaccine HB-ATV-8 represents a potential preventive strategy against the development of atherosclerosis and non-alcoholic fatty liver disease.

Frequency of expression of TGF-βRII and its association with sex steroid hormone receptors in ovarian serous carcinomas

Argelia Calvillo Robledo[2], Pablo Damián Matsumura[2], María José Gómora Herrera[1], Flavia Morales Vásquez[3], María del Carmen Méndez-Herrera[1], Enrique Antonio Pedernera Astegiano[1]*. Universidad Nacional Autónoma de México[1]. Universidad Autónoma Metropolitana[2]. Instituto Nacional de Cancerología[3]. Universidad Nacional Autónoma de México, Facultad de Medicina, CP. 04510 Tel. (55) 56 23 22 64, epa@unam.mx*

Abstract

Epithelial ovarian cancer, is the most common and lethal ovarian neoplasms which is frequently detected in advanced stages of the disease. Consequently, 60-70% of women presents recurrence and become less responsive to chemotherapy treatment. Ovarian carcinomas are classified according to their molecular, genetic and histopathologic features, where serous tumors are the most frequent and account for about 75% of all reported cases; and can be subdivided in Borderline tumors, Low Grade Serous Carcinomas (LGSC) and High Grade Serous Carcinomas (HGSC). Sex steroid hormones have been associated with the development of ovarian cancer; such is the case of androgens and estrogens and their receptors AR and ER, respectively; these hormones have been involved in the promotion of cell division, epithelial mesenchymal transition and inhibition of apoptosis. On the other hand, several studies indicate that progesterone and its receptor PR would have a favorable effect on the prognosis and overall survival of the patients. It has been proposed that receptor II of transforming growth factor-beta (TGF-βRII) could be interacting with AR, PR and ER alpha at various points in the TGF-β signaling cascade; this interactions potential several processes mainly related to cellular proliferation of the ovarian carcinomas, however, the relationship between these receptors is not entirely clear. The purpose of this study is to evaluate the co-expression of TGF-βRII and steroid hormone receptors AR, PR and ER alpha in ovarian serous carcinomas and their association with tumor cell proliferation. Our results show that expression of each receptor is different in serous carcinomas, AR (70%) and PR (68%) being more frequently expressed with respect to ER alpha (40%) and TGF-βRII (43%); likewise, when we evaluated tumors by degree of malignancy, a decrease in the expression of ER alpha and TGF-βRII was observed in HGSC tumors with respect to LGSC and Borderline tumors. When assessing the co-expression of the receptors, it was found that the expression frequency TGF-βRII decreases in HGSC with respect to Borderline tumors in the presence of PR and absence of AR. In conclusion, we confirm the association between the expression of TGF-βRII with PR and AR diversely in each malignancy degree of serous carcinomas.

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EVALUATION OF THE FIBROINE AND THE ALGINATE AS A BIOMATERIAL OF SCAFFOLDING FOR THE GROWTH OF CELLS FROM THE VASCULAR ESTROMAL FRACTION

Susana G. Cardenas-Ramos, Nidia K. Moncada-Saucedo, Alejandro Garcia-Ruiz, Vanessa Perez-Silos, Mayra Bazan-Alvarez, Alberto Camacho, Viktor Romero-Diaz, Ivan Marino-Martinez, Gabriel Mecott-Rivera, Lizeth Alicia Fuentes-Mera [1,2].
[1] Laboratorio de Medicina Regenerativa. Departamento de Bioquimica de la Facultad de Medicina de la Universidad Autonoma de Nuevo Leon (UANL).
Madero y Dr. Aguirre Pequeno Col. Mitras Centro sin numero. 64460, Monterrey, N.L., Mexico.
[2] +52 (81) 83294173 ext 2588; lizeth46@hotmail.com

Introduction: Skin lesions that cover a large area of the skin, require immediate coverage of the affected area to reduce risks of sepsis and facilitate tissue regeneration. A novel therapeutic alternative is tissue engineering. It has been demonstrated that silk fibroin scaffolds are a suitable substrate to improve tissue regeneration when they are cellularized. In these, the architecture of the pores in the scaffolds plays a vital role so that the cultured cells can be distributed and organized into a functional tissue. On the other hand, alginate has been widely used due to its high biocompatibility, low cost, reduced immunogenicity and its ability to form solid constructs with a homogeneous distribution of cells. Regarding the cellular component, we have selected cells from the stromal vascular fraction (SVF) since it is a component of adipose tissue rich in stem cells, fibroblasts and endothelial cells capable of promoting vascularization, a vital process for the rapid healing of skin injuries.
Methodology: The SVF cells were isolated from human adipose tissue by digestion with the NB6 collagenase. 3mm thick scaffolds of fibroin were generated by the leaching method with sodium chloride, particle size of 40-70 µm and 2% alginate by gelation with CaCl2. The quantification of cell proliferation was performed by the Alamar blue method. To evaluate the scaffold structure, histological sections stained with hematoxylin and eosin (H & E) were prepared. The ultramicrostructure and interconnectivity was analyzed by scanning electron microscopy (SEM).
Results: An alginate-based construct of reproducible size and of adequate and elastic macroscopic appearance was obtained. Regarding fibroin scaffolds; The leaching method generated a uniform scaffolding, homogeneous structure and with porosity in its macroscopic examination. Through the proliferation assay, it was observed that the alginate scaffold incorporated a greater number of cells compared to that of fibroin. By light microscopy, a closed, dense and homogeneous network in the form of a characteristic honeycomb was observed in the fibroin scaffolds; while in alginate the network presented spaces of variable size. The SEM analysis allowed us to study the ultramicrostructure of the fibroin scaffolding,showing homogenous and interconnected pore sizes.
Conclusions: A reproducible methodology for the elaboration of silk fibroin and alginate scaffolds was defined; Alginate being the biomaterial with the best qualities to support the culture and expansion of cells from the SVF. These data allow us to go forward in the design and construction of a cellular implant with applications to wound healing.

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Expression profile and subcellular localization of the Heat Shock Proteins, Hsp90α and Hsp90β, with the androgen receptor, allows to identify a patients subgroup with prostate cancer susceptible to the Hsp90 inhibition in primary cell cultures.

Rafael Castillo Negrete, Aline Kay Romero Mandujano, Ilse Mariana Hernández Flores, Miguel Angel Jiménez Ríos, Ma. Delia Pérez-Montiel Gómez, José De la Luz Díaz Chávez, Cristian Arriaga Canon, Luis Alonso Herrera Montalvo, Carlo César Cortés González
Instituto de Investigaciones Biomédicas of Universidad Nacional Autónoma de México and Instituto Nacional de Cancerología, México. C.P. 14080. ccortes@biomedicas.unam.mx

Introduction

Prostate Cancer (PC) remains a significant cause of mortality, having the first place in incidence and mortality in men in Mexico. The PC is a tumor dependent on the activation of the androgen receptor (AR), belonging to the group of steroid nuclear receptors, and its activation depends on the binding to its ligand, androgens, functioning as a transcriptional factor. The first line treatment for most patients with PC is hormone deprivation therapy, being effective in a median of 1 to 2 years, and later developing an independence to androgens, called castration resistant prostate cancer (CRPC), characterized by overexpression and independence of RA to androgens.

In PC, the Heat Shock Proteins, Hsp90α and Hsp90β, are molecular chaperones that facilitate cellular homeostasis by regulating the folding, stabilization and transport of the RA. An increase in the expression of Hsp90 tumors of CP correlated with the degree of Gleason Grade and TNM staging. The inhibitor of Hsp90,17-DMAG inhibits the binding site of ATP and prevents its role as molecular chaperone. It is necessary to search for new therapies targeting this disease, where the isoforms Hsp90α and Hsp90β could have a differential role that dictates the progression or response of PC.

Objective

To study the expression and localization profile of the isoforms Hsp90α and Hsp90β in primary cell cultures of patients with prostate cancer, and determine *ex vivo*, its association with the susceptibility to the Hsp90 inhibition.

Results

In primary cell cultures derived from patients with prostate cancer (PCCPC) treated with the inhibitor of Hsp90, 17-DMAG, Hsp90α downregulation was observed with an increased susceptibility to the Hsp90 inhibition determined by Western blot. While Hsp90β expression did not change significantly.

The CCPC that exhibited downregulation of Hsp90α after the treatment with 17-DMAG, the Androgen Receptor lost its nuclear localization determined by Immunofluorescence

Conclusions

This preclinical study allows to identify a subgroup of *ex vivo* cultures that can be benefited with 17-DMAG inhibition therapy, through Hsp90 and Hsp90β expression profile with RA subcellular localization.

Expressions of metalloproteinases associated with p-ERK in ovarian tumors.

Castillo Sánchez Rocío[1], Gómora María José[1], Morales-Vásquez Flavia[2], Pedernera Enrique[1], Hernández Martínez Azucena[3], Méndez Herrera María del Carmen[1]*.
[1]Departamento de Embriología, Facultad de Medicina, Universidad Nacional Autónoma de México UNAM.
[2]Instituto Nacional de Cancerología INCan. 3Hospital Militar de Especialidades de la Mujer y Neonatología. Secretaria de la Defensa Nacional SEDENA. CdMx, México. Universidad Nacional Autónoma de México, Facultad de Medicina, CP. 04510 Tel. (55) 56 23 22 64, carmenmh2001@yahoo.com.mx*

Abstract

The ovarian cancer is a leading cause of death of gynecologic malignancies. The 90% of the cases correspond to epithelial ovarian cancer (EOC), which is presented in five histological subtypes: high-grade serous carcinoma, low grade serous, endometrioid, mucinous and clear cell. Extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) are members of the super family of the mitogen-activated protein kinases, MAPKs their activation carries substrate phosphorylation, including cytoskeletal proteins and transcription factors, stimulate the growth and the spread of tumor cells. There is evidence that the treatment with MAPK inhibitors suppress significantly the migration and invasion, as well as the expression of matrix metalloproteinases (MMPs) in ovarian cancer cells. The aim of this study was to evaluate association of the phosphorylated proteins ERK1/2 and MMP-2 and 9 in ovarian carcinoma. The expression of ERK1/2 and MMP-2 and 9, was evaluated in 82 surgical pieces formaldehyde- fixed and embedded in paraffin using immunohistochemistry technique. The results showed that ERK1/2 phosphorylated are strongly associated with MMP-2 expression in epithelium and stroma $p<0.001$ and $p=0.014$, respectively. The association of MMP-9 with *ERK1/2* phosphorylated was not observed. The analysis of EOC by histological subtypes, showed p-ERK1/2 and MMP-2 are associated in the endometrioid ($p=0.025$) and mucinous ($p= 0.015$) tumors; also a negative association between p-ERK1/2 and MMP-9 was observed in the stroma of borderline serous tumors *(*$p=0.02$). In conclusion, there is a clear association between activation of MAPKs signaling pathway evidenced through the phosphorylated ERK1/2 with the expression MMP-2, but absent for MMP9, this observation is differentially observed in EOC histological subtypes.

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Biological implications of the proteins and microRNAs contained in hepatocellular carcinoma cells-derived extracellular vesicles

Luis Alberto Castro Sánchez1,2*, Jessica Lizeth Serrano Dávila1, Kenia Alejandra Gómez Reyes1, Francisco Alfredo Molina Pelayo1, Rosendo García Carrillo1, Jesús Monroy Rodríguez1, Andrómeda Liñán Rico1,2, Mónica Sierra Martínez3, Enrique Chávez Jiménez4
1Centro Universitario de Investigaciones Biomédicas, Universidad de Colima. Avenida 25 de Julio 965, Colonia Villa San Sebastián, Colima Col., México, C.P 28045.
2Consejo Nacional de Ciencia y Tecnología (CONACyT).
3Hospital Juárez de México.
4Instituto de Fisiología Celular, Universidad Nacional Autónoma de México.
*Corresponding author: luis_castro@ucol.mx, Tel. (312)-3161000 Ext. 47559.

Hepatocellular carcinoma (HCC) is the most common histologic type of primary liver cancer worldwide. Besides, this pathology represents the sixth most frequent neoplasia and the second cause of cancer mortality in the world. The hepatic tumor is the consequence of a cell communication process that are established to generate a favorable environment for the HCC development. One of the elements that have been proposed in this process are the extracellular vesicles (EVs), which are increased in the blood of patients with this neoplasia type. The EVs are defined as spherical proteolipids of double membrane and classified according to their origin and size in three different groups: exosomes, microvesicles and apoptotic bodies. Most of the HCC tumors are constituted by malignant cells with various differentiation grades, which can secrete EVs that are contained dissimilar molecular components and could influence in the cancer progression. Therefore, the present work evaluated the biological effects of the EVs secreted by HCC cells with diverse differentiation degrees on the viability, proliferation and migration of tumor and non-tumor hepatic cell lines. Our results show that the EVs derived from HCC cells of well-differentiated and poorly differentiated stages induced an increase of the viability of non-tumor hepatocytes, while the EVs secreted by moderately differentiated cells promoted a decrease of viability. Moreover, the EVs liberated from hepatoma cells of all different differentiation grades stimulated an increase of hepatic tumoral cells viability. In this regard, the EVs of hepatocarcinoma cells with distinct differentiation stages encouraged the proliferation of HCC cells, as well as, of non-tumour hepatocytes. In addition, the EVs released by HCC cells promoted the migration of non-tumorous hepatocytes, while the EVs derived of well-differentiated and poorly differentiated liver cancer cells stimulated the migration of well-differentiated HCC cells. However, the EVs derived of poorly differentiated hepatoma cells induced collective migration on HCC cells of its own tumor stage. Finally, we determined the involvement of both proteins and microRNAs contained in the HCC cells-derived EVs on the proliferation and migration of tumor and non-tumor liver cell lines. Our results show a similar contribution on both proliferation and migration of the hepatic cell lines. These data suggest that molecular content of hepatic tumor cells-derived extracellular vesicles participate in the HCC development.

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Effect of acetonic extract of *Ficus* spp. leaves on the migration capacity of tumour cells MDA-MB-231.

Lorena Cayetano Salazar, Napoleón Navarro Tito, Patricia Álvarez Fitz, Macdiel Emilio Acevedo Quiroz, Ana Elvira Zacapala Gómez, Dinorah Nashely Martínez Carrillo, Miguel Ángel Mendoza Catalán.
Autonomous University of Guerrero, Faculty of Biological Chemical Sciences. Av. Lázaro Cárdenas S/n, University City, Chilpancingo, Guerrero, Mexico. CP 39070. mglmendoza7@gmail.com
Key words: *Ficus*, cell migration, breast cancer.

Introduction

Breast cancer represents one of the main causes of deaths at world level, the survival after surgical treatment, chemotherapy or radiation therapy remains deficient, for its high rates of recurrence and metástasis, being this event the main cause of death, where migration and invasión cell play a central role in its propagation2; for this reason there is a critical need to seek new safer alternatives1. Worldwide reports indicate the biological activity of the genus *Ficus* as a potential source for the development of new anticáncer agents3. Mexico counts with various endemic species of this genus, however, no report exists on its biological activity.

Objective

To evaluate the effect of the acetonic extract of *Ficus* spp. leaves on the cell migration capacity MDA-MB-231

Methodology

The cell line MDA-MB-231 was cultivated to evaluate the migration capacity by means of the wound closure and the transwell assay, using the acetonic extract of *Ficus* spp. leaves at concentrations of 5, 10 and 20 µg/ml in the presence and absence of leptin, at 24 and 48 hours of treatment, with respect to the vehicle (DMSO) and the positive control of the stimulus with leptin.

Results and discussion

The results show that the acetonic extract of *Ficus* spp. leaves decreased the capacity of migration in 20 to 30% compared to the cells treated with the vehicle of the extracts (DMSO) at 24 and 48 hours of treatment, in a dose dependent manner, being more evident at concentrations of 10 and 20 µg/ml; interestingly, the acetonic extract of *Ficus* spp. leaves decreased cell migration capacity by 40 to 50% even in the presence of leptin stimulus which is known to promote the migration of MDA-MB-231 cells. The effect that we observed may be due to a decrease in the secretion of metalloproteinase-2 and 91; however, the mechanisms of action will be evaluated later.

Conclusions

The acetonic extract of *Ficus* spp. leaves decreased motility of cells MDA-MB-231, in a dose-dependent manner, even in the presence of the stimulus with leptin, being more evident at concentrations of 10 and 20 µg/ml.

Acknowledgments

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The expression the receptor of PRL and GH is regulated by the activation of estrogen receptor β (ER-β) of triple negative breast cancer cells.

Cecilia Cruz Neria, María Julieta López Cuevas, Eduardo Monjaraz Guzmán; Laboratorio de Neuroendocrinología, Instituto de Fisiología, Benemérita Universidad Autónoma de Puebla (BUAP). Avenida 14 Sur 6301, Col. San Manuel, CP 72570, Puebla, Pue. Tel. (222) 2 29 55 00 ext. 7311
Email: cbel_10@hotmail.com.

BACKGROUND

Triple negative breast cancer represents 15 to 20% of all cases of breast cancer, presents the most aggressive phenotype and the worst prognosis of survival. These tumor cells are characterized by being negative for the expression of ERα, PR y HER2, so that there is no efficient pharmacological treatment so far. Recently, it was described that these tumor cells expressed ERβ, unknowing until now functional relevance. Multiple clinical evidences show that the expression of PRL and GH are positively related to the development and tumor progression. Therefore, the purpose of the present work is to investigate whether ERβ activation influences the expression levels of PRL/ PRL-R and GH/GH-R complexes in triple negative breast cancer cells, as well as their capacity proliferative and migratory.

METHODS

The MDA-MB 231 cell line was employed as an experimental model, which was maintained in an estrogen-free culture medium, and in the presence of DPN, a specific agonist of ER-β. By end-point RT-PCR assays, the expression levels of the mRNA encoding the PRL and GH receptors were detected. In order to evaluate the proliferative capacity of the MDA-MB 231 cells maintained in the presence of DPN, we carried out incorporation assay of BrdU, and to evaluate the migratory capacity, we carried out assay in transwell chambers.

RESULTS

The tonic activation of ERβ by DPN regulates the level of expression of the mRNA that codes for the receptors to PRL and GH, as well as the proliferative and migratory capacity of the MDA-MB 231 cells, which will help to understand a little more about the molecular mechanisms involved in tumor progression and development, and will allow, in the medium and long term, to propose new pharmacological targets for the efficient treatment of triple negative breast cancer.

Proteomic analysis of serum patients with insulin resistance

Walter David Cruz Pineda[1], Olga Lilia Garibay Cerdenares[2,4], Sergio Manuel Encarnación Guevara[3], Magdalena Hernández Ortiz[3], Gabriel Martínez Batallar[3], Marco Antonio Leyva Vázquez[1], Mónica Ramírez Ruano[1] e Isela Parra Rojas[1]*.
[1] Laboratory of Obesity and diabetes Research, Faculty of Chemical and Biological Sciences, University Autonomous of Guerrero.
[2] Laboratory of Molecular Biomedicine, Faculty of Chemical and Biological Sciences, University Autonomous of Guerrero
[3] Laboratory of Proteomics, Center of Genomic Sciences-UNAM.
[4] CONACyT-University Autonomous of Guerrero
* Corresponding author: iprojas@yahoo.com; Ciudad Universitaria, Edificio C. Tel.: 7473998617

Key words: insulin resistance, serum, Proteomics

Insulin resistance is a condition in which the physiological activity of the hormone on target cells is impaired. The onset of this disease is commonly associated with the presence of others such as obesity and chronic low-grade inflammation, however, there is evidence that insulin resistance can be favored even in non-diabetic individuals with normal body weight, although the mechanisms involved in the process are not entirely clear. Blood serum is a type of sample easily accessible and, in addition to containing the proteins of the blood, is the secretome of all cells and tissues of the body. These characteristics are favorable for the use of serum in the search for proteins that are expressed differentially when comparing samples from individuals with presence and absence of insulin resistance and thereby identifying those that are potentially candidates as biomarkers of the disease. In this study, 24 serum samples grouped in 4 pools corresponding to children and adults with and without insulin resistance were analyzed, through a semiquantitative analysis with PDQuest Software v.8.0.1 trough 2D electrophoresis profile and the identification of the proteins by MALDI-TOF mass spectrometry. Ten proteins with statistically significant differential expression were identified and their function was determined by in silico analysis, subcellular localization and the direct interaction of 8 of them. In the same way, it was determined that some of these proteins are involved in the regulation of processes such as oxidative stress and endoplasmic reticulum stress both in pancreatic tissue and insulin target tissues, which have been associated with the emergence and development of resistance to insulin.

Protein levels of pIRE1, ATF6, pPERK and BiP (Unfolded Protein Response mediators) in keratoconus

Francisco Emmanuel Cruz Trápala, Luz María Bautista Velasco, Raquel Guerrero Alba, Eduardo Emmanuel Valdez Morales, Sugela Susana Blancas Zugarazo
Departamento de Cirugía, Centro de Ciencias de la Salud, Universidad Autónoma de Aguascalientes
Av. Universidad No. 940, Cd. Universitaria, CP 20131, Aguascalientes, Ags. Edif. 107. Tel. 01 (449) 910 8000, Ext. 8432. E-mail: ssblancas@gmail.com

Unfolded Protein Response (UPR) pathway has an important role in cellular homeostasis. This pathway is associated to unfolding protein accumulation in the Endoplasmic Reticulum in the cell. The UPR regulate the protein production to avoid the protein accumulation. There are three main regulators in the UPR, which are IRE1, ATF6 and PERK, and a three-way master regulator, BiP. Activation of UPR pathway has been associated with many diseases including diabetes and cancer. Particularly in ocular diseases, this pathway is activated in pathologies as glaucoma, cataract and Fuchs' endothelial dystrophy, but it is unknown its possible activation in keratoconus. In this work, we explored the UPR pathway role in keratoconus disease.

The human tissue was obtained from the *Eye and Tissue Bank of Aguascalientes*, according with ethical committee of UAA and the Eye-Bank. Eye-Bank donated us the keratoconus corneas from patients after penetrating keratoplasty as well as the remains of the healthy corneas used in the transplant. The protein *in situ* levels of the UPR mediators were measured by immunofluorescence assays in healthy and keratoconus corneas.

When we explored the *in situ* protein levels of the UPR regulators, the BiP and ATF6 levels were lower in keratoconus than healthy corneas, in both corneal layers epithelium and stroma. The pPERK levels in the corneal epithelium were lower in keratoconus than healthy corneas, but pIRE1 showed the same levels in keratoconus and healthy corneas. These results suggest that UPR pathway could be associated with keratoconus pathogenesis.

Differential expression of proteins in an atypical presentation of Autoimmune Lymphoproliferative Syndrome

[1]Dulce Maria del Carmen Delgadillo-Álvarez, [1]Misael Domínguez-Fuentes, [2]Adriana Ivonne Céspedes-Cruz, [2]Maritza Zeferino-Cruz, [2]Mariel López-Nogueda, [1]Lorena Ramírez-Reyes, [1]Emmanuel Ríos-Castro and [1]*José Tapia-Ramírez.
[1]Unidad de Genómica, Proteómica y Metabolómica. Laboratorio Nacional de Servicios Experimentales (LaNSE). Centro de Investigación y de Estudios Avanzados (CINVESTAV). [2]Reumatología Pediátrica, Unidad Médica de Alta Especialidad (UMAE) Centro Médico Nacional La Raza Hospital General.
*Av. Instituto Politécnico Nacional # 2508, Col. San Pedro Zacatenco, C.P. 07360 Ciudad de México. Tel. 57473800 ext. 5368. jtapia@cinvestav.mx

Programmed cell death, or apoptosis, plays a critical role in regulating lymphocyte development and homeostasis. Apoptosis defects may contribute to abnormal lymphocyte accumulation, autoimmunity and lymphoid malignancy. Autoimmune lymphoproliferative syndrome (ALPS) is a rare disease characterized by chronic massive, nonmalignant lymphoadenopathy and splenomegaly; expansion of a normally rare population of T cells bearing ab-antigen receptors but lacking both CD4 and CD8 coreceptors ($\alpha\beta$ double-negative T cells, $\alpha\beta$DNTs). ALPS has been defined as a defect in the lymphocyte apoptotic pathway and is associate with inherited mutations in the FAS gene (60-70%) in the germinal line while 10% are somatic mutations in the same gene. With less frequency, the mutation is in the Fas ligand (FAS-L) and in the caspase 10 genes (<1% and 2-3% of the patients, respectively). When mutations are detected in these genes, the patients are classified as ALPS-FAS, ALPS-LG or ALPS-CASP10. However, 20-30% of the patients clinically diagnosed do not present any known mutations (ALPS-U). Until now, the definitive diagnosis of ALPS is based in clinical aspects, defective lymphocyte apoptosis and mutation in the FAS, FASLG and CASP10 genes. We report here the case of a 10 years old girl with a possible diagnostic of ALPS. Diagnosis of rheumatologic disease was discarded and the patient shows hepatoand splenomegaly, autoimmune hepatitis, cytopenia, presence of negative antibodies and increase in the number of double negative CD4/CD8 cells (1.8). However in spite of these data, in a genomic analysis no mutations were found in the FAS and CASP10 genes. In order to go further in the knowledge of ALPS we have initiated the study of the proteome of this patient. As it is known, proteomics can provide significant biological information for many biological problems such as those derived from a pathological process. The information achieved will provide us valuable elements to make a more integral diagnostic of ALPS and also, others autoimmune diseases.

Infections in Pediatric Patients with Neoplasia and Neutropenia of the Instituto Estatal de Cancerología "Dr. Arturo Beltrán Ortega" Acapulco, Guerrero

Sacnite del Mar Díaz González1, Sallaly Jassel Abarca Rentería1, Miguel Angel Díaz Alday2, Ana Bertha Rivera Ramírez1, Evelin Margarita Morales Flores1, Margarita Yaravit Kuri Guinto1, Mónica Virginia Saavedra Herrera1, Libia Monserrat Campos Olguin1, Marco Antonio Jiménez López1
1Instituto Estatal de Cancerología —DrArturo Beltrán Ortega" 2Instituto Tecnológico de Acapulco
Adolfo Ruiz Cortines 128, Acapulco de Juárez, Guerrero, México
045 7442088080 - sacnimar@gmail.com

INTRODUCTION

The treatments against neoplasias in pediatrics represent a great advance in the approach of these pathologies, however, therapeutic strategies have important side effects. The suppression of the bone marrow produces intermittent periods neutropenia of different severity and duration. Patients with cancer, neutropenia and fever constitute the group with the highest risk and infectious urgency, since the infections in these patients may not reveal clinical signs and may be rapidly evolving and fulminating. Therefore, our objective was to identify the microorganisms that most frequently infect pediatric cancer patients with neutropenia in the Instituto Estatal de Cancerología —DrArturo Beltrán Ortega".

METHODOLOGY

A prospective observational study was performed on oncopediatric patients from September 2013 to April 2014. Blood cultures, pharyngeal, nasal, abscess and catheter exudates were processed. The blood cultures were carried out in the Pediatric Hemoculture bottles (Bio Rad, Mexico, DF). The samples were seeded in 50 ml of Mac Conkey agar, Blood Base and Staphylococcus 110, incubated at 37$_{o}$C for 24 and 48 hours, then microbiological identification was performed.

RESULTS

From 2013 to 2014 the area of pediatrics treated 18 different types of cancer, the most frequent being lymphoblastic acute leukemia (48.98%). The general prevalence of infections in oncopediatric patients in the period studied is 14.2%, the most frequent microorganism is *Staphylococcus aureus* (37.0%). Of the patients with neutropenia determined by a clinical laboratory at 38.5%, an infection based on clinical focus was determined, 17.9% infection with a microbiological basis and 43.6% presented fever without an identified focus.

CONCLUSION

Considering the immunosuppression status of oncopediatric patients, it is important to determine the microorganisms that frequently cause sepsis and infections of catheters and anatomical sites. In the IECAN, based on this evidence, in the oncopediátria service surveillance strategies have been carried out to preserve the infant receiving both chemotherapy and transfusion support.

ATORVASTATIN AND ROSUVASTATIN: UNDESIRABLE SIDE EFFECTS OF HIGH DOSES IN HYPERCHOLESTEROLEMIC RODENTS

Juan Cuauhtémoc Díaz Zagoya[1]*, Isela Esther Juárez Rojop[2], Alma Mileira Zetina Esquivel[2], Andrés Eliud Castell Rodríguez[1], Jorge Luis Blé Castillo[2], Rodrigo Miranda Zamora[1].
[1]División de Investigación, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México. [2]División Académica de Ciencias de la Salud, Universidad Juárez Autónoma de Tabasco, Villahermosa, Tabasco, México.
*Juan C. Díaz Zagoya. Departamento de Bioquímica, División de Investigación, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México, CP 04510, zagoya@unam.mx

Statins are the choice drugs for the first and second prevention of cardiovascular disease (CVD). They are competitive inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. Statins are extensively used in patients with high serum cholesterol levels to control this recognized CVD risk factor. As any pharmaceutical compound, statins are not exempted of undesirable side effects specially on the liver, when high therapeutic doses are employed or when patients are intolerant to this type of drugs. We used Wistar rats to evaluate atorvastatin (ATV) and CD-1 male mice to evaluate ATV or rosuvastatin (RVT) side effects on the liver of hypercholesterolemic animals. ATV was given to rats 3.25, 6.5 and 13 mg/Kg/day, 30 days. Serum cholesterol was not modified but triacylglycerol decreased with treatment. A fatty liver was produced in all groups with a fat gradient distribution from portal vein to central veins. An uncoupling effect was also present in hepatocyte mitochondria. In hypercholesterolemic mice, ATV or RVT was administered, 0 to 400 mg/Kg/day (n=6). This combination produced a dose-dependent mortality with both statins. In other experiment we administered RVT 1, 2.5, 5, 10 and 20 mg/Kg/day, to hypercholesterolemic mice, evaluating mitochondrial function and microscopic morphology of the liver. Our results showed that only the 1 mg/Kg/day dose was not harmful to the liver evidenced by the similar cellular architecture and the respiratory coefficient (3.56 nmol O_2/mg protein/min) in the treated animals as in controls. In conclusion, statins constitute a powerful tool for serum cholesterol control, but high doses treatments could be harmful specially for those individuals intolerant to this kind of drugs. It is also evident that there are species differences in response to high statin dose treatments in hypercholesterolemic rodents.

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Hypoglycemic effect of the aqueous extract of Galeana leaves (*Spathodea campanulata*) in diabetic rats.

María del Socorro Escamilla Barrera[1]*, José Miguel Cervantes Alfaro[2], Luz Torner Aguilar[3], Carlos Cortés Penagos[4], Marcia Ivette Gauthereau Torres[5], Aarón Vázquez Hernández[6] and Bertha Fenton Navarro **[1]
[1] Glycobiology Laboratory, [2] Neurosciences Laboratory, [4] Cell Biology Laboratory, [5]Pharmacology Laboratory. Postgraduate Studies Division, Faculty of Medicine and Biological Sciences ~~-~~Dr. Ignacio Chávez", Michoacán University of Saint Nicholas of Hidalgo (*UMSNH*), Morelia, Michoacán, Mexico [3]Michoacán Biomedical Research Center, IMSS, Michoacán. [6]Specialties Hospital, CMN SXXI, IMSS, Mexico City, Mexico. *qfbsocoumich@gmail.com, **bertha00_mx@yahoo.com. Tel: 52-443-3120014 ext. 234

Introduction: Diabetes and its complications belong to chronic degenerative diseases that constitute a global health problem due to the deterioration in the life quality of individuals, in addition to the social and economic implications associated with this pathology. The plants synthesize a great variety of secondary metabolites of which there are scientific reports that confirm their biological activity in pathologies such as diabetes mellitus, for which they represent an alternative and coadjutants in the treatments against this disease. **Objective**: To evaluate the hypoglycemic effect of Galeana aqueous extract (*Spathodea campanulata*) in a rat type 2 diabetes mellitus model.

Material and methods: Aqueous extract of Galeana leaves (5 to 5000mg / Kg). Acute evaluation of hypoglycemic capacity of aqueous extract using glucose tolerance curves in type 2 diabetic male Wistar rats (from 100mg) randomly distributed in the following groups (n=6): Healthy controls, Diabetics with saline solution, Diabetics with aqueous extract of Galeana leaves and Diabetics with Metformin; the induction of diabetes mellitus type 2 was performed by oral administration of 60% fructose.

Results: The hypoglycemic results of Galeana aqueous extract from the different doses where: 5 mg/kg of 10.98%, 25 mg/kg of 7.6%, 50 mg/kg of 5.96%, 150 mg/kg of 14.08%, 300 mg/kg of 17.36%, 2000 mg/kg of 7.99% and 5000 mg/kg of 11.73%, in addition, as controls, the rats treated with saline showed a reduction of 0% and those treated with metformin showed a reduction of 26.36%.

Conclusion: The concentration of 300 mg/kg was established as the optimal hypoglycemic dose of the aqueous extract of Galena leaves showing the highest reduction percentage in the blood glucose of the diabetic animals.

Keywords: diabetes, hypoglycemic, aqueous extract.

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Effect of *Spirulina maxima* in rats treated with fried oil as prooxidant.

María Teresa Espinosa García, Miriam Sánchez Rodríguez, Marco Antonio Juárez Oropeza and Patricia Victoria Torres Durán
mteresa@unam.mx
Depto. de Bioquímica, Facultad de Medicina, UNAM apartado Postal 70-159 CP 04510 Ciudad de México; teléfono 56232169.

The Mexican population prefers to consume fried foods preferably, because these provide flavor, color and texture. However, the continuous cooking of the oil used during this process, (180 ^{0} C), humidity and the air leads to the formation of oxidized fatty acids, polar compounds or potentially toxic polymerized products. It is also known that the consumption of fried foods contributes to the appearance of diseases associated with dyslipidemia, oxidative stress and inflammatory processes such as obesity and atherosclerosis (1).

Previous reports have shown that *Spirulina maxima* has antioxidant, hepatoprotective and decreased serum triacylglycerols effects, an effect that has been related to the presence of phycobilins and other antioxidants present in this cyanobacterium.

The objective of this work is to show the protective effect of the aqueous extract of *Spirulina maxima* (Sme) on lipemia, when the rats were fed with fried oil.

Male Wistar rats, were divided into six groups: Sme, unfried oil, fried oil, and unfried or fried oil combinations plus Sm. The treatment was administered by orophageal probe (oils and Sme). The food and water were ad libitum.

Preliminary results showed that consuming the fried oil for 30 days produced an increase in retroperitoneal fat of 60% in relation to the control rats, glucose, triacylglycerols, and serum MDA concentrations were increased by 30% compared to controls, it was also observed that the group treated with Sm plus fried oil presented a decrease of approximately 20% of the parameters measured in relation to the group that was administered fried oil.

These results suggest that the aqueous extract of *Spirulina maxima* reduces lipid storage in addition it shown antioxidant effect in this study model.

(1) Zhou et al. Lipids in Health and Disease (2016) 15:86 p 1-11

TLR4 activation contributes to mesenchymal phenotype in U87 cells, favoring their metastatic potential

Miriam Fernández-Gallardo[1], Eduardo Monjaraz-Guzmán[2], Diego Ricardo Félix Grijalva[3], José Rodolfo Delgado Lezama[1]
[1] Departamento de Fisiología Biofísica y Neurociencias, CINVESTAV-IPN.
[2] Instituto de Fisiología, BUAP.
[3] Departamento de Biología Celular, CINVESTAV-IPN Av. Instituto Politécnico Nacional 2508, Col. San Pedro Zacatenco, Del. GAM C.P. 07360 México D.F. Teléfono 5747 3800 (Oficina/Lab. 5128),
Email: mirifer11@hotmail.com

Glioblastoma multiforme (GBM) is one of the most aggressive primary brain tumors, due to the lack of efficient therapeutic strategies it has one of the highest mortality rates among all types of cancer. One of the main characteristics of the GBM is its high metastatic potential to invade surrounding tissue, observing a positive correlation between the invasive capacity of the tumor and the mesenchymal phenotype of the cells. It is unknown until now which signal or signals from the tumor microenvironment influence the mesenchymal phenotype of GBM. TLRs are expressed on all cells in the CNS and not only microglia, However, what would the role of TLRs in glioblastoma be?. In the present study, we will evaluate if the activation of TLR4 affects the expression of molecular markers characteristic of the mesenchymal phenotype (Snail1, Snail 2, Twist, Zeb1, vimentin and E-cadherin), as well as its proliferative and migratory capacity in the U87 cells.

Methods: U87-MG glioblastoma cells were obtained from American Type Culture Collection. By end-point RT-PCR assays, the expression levels of the mRNA encoding the Snail1, Snail 2, Twist, Zeb1, vimentin and E-cadherin were detected. In order to evaluate the proliferative capacity of the U87-MG cells maintained in the presence of LPS, we carried out incorporation assay of BrdU, and to evaluate the migratory capacity, we carried out assay in transwell chambers.

Results: The TLR4 activation by LPS regulates the level of expression of the mRNA that codes for the molecular markers characteristic of the mesenchymal phenotype, favoring its metastatic potential, specifically, its proliferative capacity and its migratory capacity

PaDef defensin from avocado (*Persea americana* var. drymifolia) is cytotoxic to K562 chronic myeloid leukemia cells through extrinsic apoptosis

Luis Jose Flores-Alvarez[1], Jaquelina Julia Guzman-Rodriguez[1], Rodolfo Lopez-Gomez[2], Rafael Salgado-Garciglia[2], Alejandra Ochoa-Zarzosa[1], Joel E. Lopez-Meza[1*]
[1] Centro Multidisciplinario de Estudios en Biotecnologia, Facultad de Medicina Veterinaria y Zootecnia, Universidad Michoacana de San Nicolas de Hidalgo, Km 9.5 Carretera Morelia-Zinapecuaro, Posta Veterinaria, C.P. 58893, Morelia, Michoacan, Mexico. [2]Instituto de Investigaciones Quimico-Biologicas, Universidad Michoacana de San Nicolas de Hidalgo, Ciudad Universitaria, Edificio B3. Autor para correspondencia: jelopezm@hotmail.com

Plant defensins, a group of antimicrobial peptides, show selective cytotoxicity toward cancer cells. However, their mechanisms of action remain poorly understood. Here, we evaluated the cytotoxicity of PaDef defensin from avocado (*Persea americana* var. drymifolia) on K562 chronic myeloid leukemia cells and analyzed the pathway involved in the induction of cell death. The defensin PaDef was not cytotoxic against human peripheral blood mononuclear cells; however, it was cytotoxic for K562 cell line (IC_{50} = 97.3 µg/ml) activating apoptosis at 12 h. PaDef did not affect the mitochondrial membrane potential (ΔΨm), neither the transmembranal potential or the release of intracellular calcium. Also, PaDef induced gene expression of caspase 8 (~2 fold), TNF-α (~4 fold) and TNFR1 (~10 fold). In addition, the activation of caspase 8 was detected at 24 h, whereas caspase 9 activity was not modified, suggesting that the extrinsic apoptosis pathway could be activated. In conclusion, PaDef induces apoptosis on K562 cells, which is related to the activation of caspase 8 and involves the participation of TNF-α, which is a novel property for a plant defensin.

"Differential expression profile of heat shock proteins of 90kDa, Hsp90□ and Hsp90□, during the formation of 3D spheroids of hormone-sensitive and hormone-resistant prostate cancer cells"

Ilse Mariana Flores Hernández, Brenda Larissa Calvo Hernández, Vilma Araceli Maldonado Lagunas, Alejandro López Saavedra, Norma Araceli Bobadilla Sandoval, Luis Alonso Herrera Montalvo, Carlo César Cortés González.
Instituto de Investigaciones Biomédicas of Universidad Nacional Autónoma de México and Instituto Nacional de Cancerología, México. C.P. 14080 ccortes@biomedicas.unam.mx

Introduction. Clinical progression in Prostate Cancer (PC) from a hormone-sensitive (HSPC) to hormone-resistant (HRPC) clinical stage is associated with adaptive changes of the androgen receptor (AR), leading to a sustained AR signaling. In clinical practice, multiple therapies have been developed to target signaling pathways including AR in PC, where heat shock protein 90kDa inhibitors have exhibited promising results.

However, studies have suggested that some Hsp90 inhibitors could act selectively over Hsp90α and Hsp90β isoforms and could affect differently the expression and activity of Hsp90 isoforms and their client proteins. Our research group, have demonstrated previously that Hsp90α and Hsp90β regulate the AKT protein in an opposite manner. In this context, during progression of HSPC to HRPC, Hsp90 isoforms could play a differential role over AKT protein and determine the adaptive changes of AR in tumorigenesis.

Objective. To determine the Hsp90α and Hsp90β expression during the formation of 3D spheroids of hormone-sensitive and hormone-resistant prostate cancer cells.

Methods. 3D-spheroids were achieved with two PC cell lines: PC3 (androgen resistant, AR-), LNCaP (androgen sensitive, AR+), and a primary culture derivate from prostate cancer patient (PCPC, AR+). The 3D spheroids formation was confirmed by phase-contrast and LightSheet Microscopy. Cell viability and 17-DMAG susceptibility were monitoring by fluorescent microscopy. The susceptibility to Hsp90 inhibition was determine by size and cytoarchitecture of 3D-spheroids employing time-response curves with 17-DMAG (Hsp90 inhibitor), [250nM]. Hsp90α and Hsp90β expression profile was evaluated by western blot and immunohistochemistry assays.

Results. Both hormone-sensitive and hormone-resistant prostate cancer cells (PC3 y LNCaP, respectively) and the primary culture (PCPC) had the ability to form spheroids. Hsp90 inhibition had different effects on 3D-spheroids formation: a) The ability to form spheroids were decreased in the PC3 cell line, in the same way as the expression of Hsp90α b) In the LNCaP cell line, the expression of Hsp90α and the capacity to form 3D-spheroids were increased, although the distinguishing cytoarchitecture was lost. c) Finally, the 17-DMAG treatment resulted in a spheroid formation delay, and a peak of Hsp90α levels at 48h in PCPC. On the other hand, the expression of Hsp90β remained constant in all the three cell lines with and without treatment.

Conclusion. Hsp90α down-expression caused by Hsp90 inhibition was associated with a loss of spheroid formation while its up-regulation increases the capacity of 3D-spheroid conformation although its cytoarchitecture was modified. The sensibility to Hsp90 inhibition was related with an aggressive phenotype along with the hormone-resistant prostate cancer cells probably because its increase dependence to Hsp90. On the other hand, PC spheroids possess the ability to maintain Hsp90β expression.

Differences on vascular reactivity and RNAm expression of Renin-Angiotensin and Kinin-Kallikrein systems components on acute and chronicmyocardial infarction in male and female Wistar rats

PhD. Jazmín Flores Monroy[1], M.Sc Diego Lezama Martínez[1], PhD. Salvador Fonseca Coronado[2], PhD. Luisa Martínez Aguilar[1,1] Laboratorio de Farmacologia del Miocardio, Facultad de Estudios Superiores Cuautitlan, Universidad Nacional Autonoma de Mexico. [2] Laboratorio de Inmunobiologia de enfermedades infecciosas, Facultad de Estudios Superiores Cuautitlan, Universidad Nacional Autónoma de México Av. 1º de Mayo s/n. Col. Sta María las Torres. Cuautitlán Izcalli, Estado de México, CP: 54740. Tel: 55-56-23-20-49. Email. jfmqfb@yahoo.com.mx

Actually relative expression of mRNA of RAS and KKS had been widely investigated on males at acute or chronic myocardial infarction; also transcriptional changes had not been related with vascular activity. The aim of this study was to evaluate the relative mRNA expression of the ACE, ACE2, AT_1, AT_2, B1 and B2 and vascular reactivity to Ang II during the evolution of myocardial infarction.

Methods: 80 male and 80 female Wistar rats were used and grouped as: 1) Sham; Coronary occlusion groups: 2) 48 h; 3) 1 week; 4) 2 weeks; 5) 3 weeks; 6) 4 weeks. To all groups the relative mRNA expression of the ACE, ACE2, AT_1, AT_2, B1 and B2 receptors of the left ventricle by real time PCR were obtained. Also infarct area, cardiac hypertrophy, systolic and diastolic blood pressure, cardiac frequency and Ang II vascular reactivity in the absence and presence of valsartan (200 nM) on aortic rings were evaluated.

Results: Both sexes showed significant increase of ACE, ACE2, AT_1, AT_2, B1 and B2 mRNA expression in males at 3 weeks of CO and females at 48 h and 2 weeks and the last expressed in less amount all components. Infarct areas were calculated between 30-40% in all groups, no changes were observed in hypertrophy index on female rats while in males there was an increase at 2, 3 and 4 weeks. A decrease in systolic and diastolic pressure in male rats at 3 weeks of CO was found. Vascular reactivity to Ang II was increased on male rats aortic rings during 4 weeks; on females, this effect is diminished since 48 h until reaching sham group values at 2 weeks of MI and the incubation with valsartan generated greater response reduction to Ang II in males than females.

Conclusion: Our findings indicate that Renin-Angiotensin and Kinin-Kallikrein systems components are over expressed on male than female rats that could be related with functional and morphological changes on development of myocardial infarction.

Association of the polymorphism rs9939609 in the *FTO* gene with type 2 diabetes in the state of Guerrero

Daicy Grisel Francisco Aguilar[1], Laura Vaneza García Pérez[1], Eugenia Flores Alfaro, Luz del Carmen Alarcón Romero, Inés Matia García, Yaneth Castro, Linda Anahí Marino Ortega *José Ángel Cahua Pablo.
[1]Investigation Laboratory in Clinical and Molecular Epidemiology of the Faculty of Biological Chemistry
Sciences. Autonomous University of Guerrero. AV. Lázaro Cárdenas S/N C.U. South Zone: Chilpancingo de los Bravo. C.P. 39070, * phone:7472256604 Mail: jose9@hotmail.com.

Type 2 diabetes (T2D) is a chronic disease characterized by insulin resistance, resulting in hyperglycemia with alterations in the metabolism of carbohydrates, lipids and proteins. The *FTO* gene codes for the FTO protein and it has been proposed that it regulates the expression of ghrelin an orexigenic hormone through the methylation of ghrelin mRNA and that the overexpression of FTO in carriers of the risk allele has a decrease in the methylation of ghrelin mRNA and higher expression predisposing to greater energy consumption and obesity. Within the polymorphisms in the *FTO* gene that are associated with T2D, rs9939609 is found as the commonly described SNP, located in intron one. Objective. Evaluate the association of the rs9939609 polymorphism in the *FTO* gene with development of type 2 diabetes in the population guerrerense. Methodology. A genetic association study was carried out between cases (T2D) and controls (without T2D), men and women from 30 to 65 years old from the state of Guerrero. The biochemical parameters were determined and the peripheral blood DNA was extracted using the rapid non-enzymatic technique. The determination of the genotypes was carried out by real-time PCR with the System 7500 team from Applied Biosystems. Both the genotypic and allelic frequencies of the studied population were determined. As well as, the Hardy-Weinberg equilibrium, ORs and confidence intervals were evaluated. The statistical analysis was performed with the software STATA v.13. A value of $p < 0.05$ was considered significant. Results. The frequencies of the genotypes were 2.4% for ancestral homozygote (TT), 29.2% for heterozygous (TA) and 68.4% for homozygous variant (AA). Regarding the allelic frequencies for the ancestral allele T was 17%, while for the allele of risk A it was 83%.

The carriers of the AA genotype had high glucose concentrations compared to noncarriers ($p=0.022$), however, no differences were found with any other clinical parameter. It was found that the AA genotype of rs9939609 in the *FTO* gene is associated with the development of T2D OR=1.97 (95% CI 1.1-3.4) ($p=0.015$).

Conclusions It was found that the carrier of the AA genotype of rs9939609 in the *FTO* gene is associated with the development of T2D in the population Guerrerense.

Key words: Type 2 diabetes, *FTO* gene, rs9939609, BMI, Obesity.

Evaluation of arginase activity in patients with Diabetes from the Yanga General Hospital in Córdoba, Veracruz, Mexico.

Moisés Franco Rodríguez, Silvia Garrido Llanos, Blanca Estela Trejo Sánchez, Adelma Escobar Ramírez, Carlos Javier López Victorio, José Arnold González Garrido.
Hospital General Córdoba, Veracruz. Km. 341.5 carretera a Veracruz zona Col. Industrial. C.P. 94690.Tel. 012717120315.
Laboratorio de Bioquímica y Biología molecular. Centro de Investigación de Ciencia y Tecnología Aplicada de Tabasco. División Académica de Ciencias Básicas. Universidad Juárez Autónoma de Tabasco. Cunduacán, México. Carretera Cunduacán-Jalpa KM. 1 Col. La Esmeralda CP. 86690. Tel. (993) 3581500 Ext. 6702, 3581573 y (914) 336 09 28. Cunduacán, Tabasco, México.
E-mail: arnold.gonzalez@ujat.mx.

Introduction. Diabetes mellitus (DM) is a chronic noncommunicable disease that increases its frequency every year, and the search for strategies that are helpful in prevention or control are the objective in various studies. DM can decrease the bioavailability of nitric oxide (NO) by various mechanisms, presenting endothelial dysfunction. NO is associated with vascular and anti-inflammatory functions. Several studies in animal models and in vitro studies suggest the participation of arginase in DM, being a direct competitor for the substrate of nitric oxide synthase (NOs), decreasing NO production. In this study, it was determined whether arginase is a marker of the progress of the disease in patients with DM.

Objective. To evaluate the activity of serum arginase in patients with DM type I and II, as a marker of disease progression.

Material and method. A cross-sectional study was carried out, in which 107 patients participated, of which 37 controls were included and 67 with DM. Fasting blood samples of 12 hr were used and the anthropometric parameters, glucose, cholesterol levels were determined and the plasma arginase activity was evaluated.

Results and conclusions. The results suggest that the activity of arginase has a role as a marker of the disease in patients with diabetes.

Keywords. Diabetes, Arginase, Biomarker.

Metatranscriptional characterization of the intestinal microbiota in health and obese with metabolic complications children

Luigui Gallardo-Becerra[1], Filiberto Sánchez[1], Adrián Ochoa-Leyva[1*].

Instituto de Biotecnología, Universidad Nacional Autónoma de México. Dirección: Av. Universidad 2001, Col. Chamilpa, CP: 62210, Cuernavaca, Morelos.
*.aochoa@ibt.unam.mx. Tel: +52 (777) 329-1614 y CDMX 562-27614

It is well known that intestinal microbiota plays a fundamental role in processes such as digestion, nutrition, pathogen colonization resistance, and in the correct development of the nervous and immune systems in the human being; whereby its functions are related with the health or disease of the host. One of the diseases related with a disbalance of the intestinal microbiota (dysbiosis) is the obesity, which is a growing health problem in the world. Specifically, in México the children population is a vulnerable group, being in the firsts places in the worldwide incidence.

Intestinal microbiota is one of the most well characterized microbial populations; however, the great majority of the studies of intestinal microbiota are focused to determine the species present in the sample (RNAr 16S profiling), or the potential genomics (metagenomics), leaving aside the transcriptional active microbiota (metatranscritomics).

The goal of this investigation is to understand how are the metatranscripcional profiles of healthy (lean) and obese with metabolic complications (OMC) Mexican children, focusing on understand the functionality of the intestinal microbiota related with the disease. To accomplish this purpose, we determine the metatranscritome of fecal samples of health (lean) and OMC groups, using simultaneous RNA-seq libraries and bioinformatic tools. Our results show that the microbiota of individuals with obesity have overexpression in genes related to the degradation of carbohydrates and fatty acids, which may be favoring the accumulation of energy in the host.

COMPARATIVE EFFECTS OF COLOMBIAN AGRAZ CONSUMPTION ON TRIGLYCERIDES AND OXIDATION MARKERS IN MEN AND WOMEN WITH METABOLIC SYNDROME

Yeisson Galvis [1]*, *Catalina Marín*[1], *Vitelbina Núñez* [1], *Juan Aristizábal* [2], *María Luz Fernández* [3], *Jacqueline Barona* [1]
[1]. Line of food and therapeutic alternatives, Ophidism Group. School of Microbiology. University of Antioquia. Medellín Colombia.
[2]. Research group in Physiology and Biochemistry-PHYSIS. School of Nutrition. University of Antioquia. Medellin Colombia.
3. Department of Nutritional Sciences, University of Connecticut, USA.
* Address: 67th street No. 53 - 108, email: yeisson.galvis@udea.edu.co

Introduction. Metabolic syndrome (MS) is a set of factors that increase the risk of cardiovascular disease. Colombia produces the fruit *Vaccinium meridionale* Swartz, known as agraz, which has demonstrated antioxidant properties that could modulate MS components. Given that there are physiological differences between men and women, such as in energy and lipid metabolism, differences between genders must be analyzed when assessing the effects of interventions to improve MS.

Objective. To evaluate comparatively between men and women with MS, the effects of Colombian agraz consumption on triglycerides and some oxidation markers.

Methodology. Double-blind, crossed over study of 12 weeks, in 26 men and 26 women with MS, paired by age and body mass index. Participants were assigned to consume daily agraz nectar or placebo during 4 weeks each. Participants consumed both treatments, separated by a washing period of 4 weeks. At the end of each treatment (agraz or placebo), serum triglycerides, oxidized Low-density lipoprotein-(oxLDL) levels and thiobarbituric acid reactive substances were measured.

Results. Triglycerides correlated positively with oxLDL in women ($r = 0.448$ $p = 0.025$), but it was statistically significant only in the placebo period, while in the agraz period this correlation was not significant. In men this correlation was not significant in any of the periods.

Conclusion. The consumption of agraz decreases the positive correlation between triglycerides and LDLox in women, but not in men with MS.

Proteomic analysis of the extracellular vesicles secreted by hepatoma cell lines: potential clinical- and pathophysiological-implications

Rosendo García Carrillo[1], Yosara Itzayana Rivera Nava[1], Francisco Alfredo Molina Pelayo[1], Jesús Monroy Rodríguez[1], Mónica Sierra Martínez[3], Enrique Chávez Jiménez[4] and Luis Alberto Castro Sánchez[1,2*]
[1]Centro Universitario de Investigaciones Biomédicas, Universidad de Colima. Avenida 25 de Julio 965, Colonia Villa San Sebastián, Colima Col., México, C.P 28045.
[2]Consejo Nacional de Ciencia y Tecnología (CONACyT).
[3]Hospital Juárez de México.
[4]Instituto de Fisiología Celular, Universidad Nacional Autónoma de México.
*Corresponding author: luis_castro@ucol.mx, Tel. (312)-3161000 Ext. 47559.

Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related death worldwide and is considered a poor prognosis neoplasia, since its incidence rate is comparable with the mortality, because of the difficulty diagnosis and treatment. Accordingly, accurate detection and differential diagnosis in early HCC stages can significantly improve patient survival. One of the factors involved in HCC development are extracellular vesicles (EVs) —defined as spherical double membrane proteolipids containing different molecular components— which are increased in the blood of patients with this neoplasia type. Therefore, the present work determined the expression of the elements involved in biogenesis and secretion of HCC cells-derived EVs with diverse differentiation grades, as well as, potential tumor markers in the protein content of the EVs. Our results show that the mRNA expression levels of the elements involved in exosomes formation: RAB7A, RAB27B, RAB25, RAB11A and RAB11B are increased in initial phases of well-differentiated HCC cells, whereas RAB7B is augmented in poorly differentiated hepatoma cell lines. Moreover, ARF-6 mRNA levels increase in primary phases of both well-differentiated and poorly differentiated HCC cells. In addition, the vesicular markers were evaluated in EVs isolated, demonstrating that Alix, Tsg-101 and CD63 protein levels (exosome markers) increase in EVs liberated by well-differentiated liver tumor cells, whereas Flotillin-1 (microvesicles marker) exhibited the highest expression in EVs secreted from poorly differentiated HCC cells. Furthermore, the final phases of both well-differentiated and poorly differentiated HCC cells secrete EVs with a greater amount of proteins, while EVs released by ending phases of poorly differentiated hepatoma cells contain a higher microRNAs concentration. These results suggest that the final phases of hepatocarcinoma cells, regardless of the differentiation stage, secrete a greater number of exosomes and microvesicles with different molecular content (proteins and microRNAs). Also, were identified 66 up-regulated proteins in hepatic tumor cells-derived EVs by LC-MS/MS proteomic analysis. In this regard, the biology systems suggested the pathophysiological functions in the tumor development and its potential clinical use possible source of neoplastic markers. Finally, we propose that 4 proteins contained in EVs could function as potential tumor markers for HCC.

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Expression of steroid sulfatase in epithelial ovarian cancer

Paulina García de Alba[1], María José Gómora[1], Miguel Ángel Almaraz[1], Flavia Morales-Vásquez[2], Carmen Méndez[1], Areli Magdaleno Becerro[1], Enrique Pedernera[1]
[1]Facultad de Medicina, Departamento de Embriología. Universidad Nacional Autónoma de México and [2]Instituto Nacional de Cancerología, Cd Mx. México.
Universidad Nacional Autónoma de México, Facultad de Medicina, CP. 04510 Tel. (55) 56 23 22 64, epa@unam.mx*

Ovarian cancer is the most lethal gynecological malignancy and third in frequency in developed countries. The most significant risk factors are early menarche, late menopause, nulliparity, infertility and polycystic ovarian syndrome. These data suggest that fluctuations of hormones in the reproductive system could be linked to the development of ovarian cancer. Recent studies have also shown an association between the development and growth of gynecological cancers with the variations of hormonal secretion. In ovarian tumors, sulfated steroid precursors can be converted into active steroid hormones with the steroid sulfatase enzyme (STS). The main function of this enzyme is to hydrolyze the sulfate groups of sulfated steroids and generate active free steroids that can bind to their corresponding hormonal receptors and consequently promote the development of tumor growth. The aim of this study was to analyze the expression of STS in the distinct histological subtypes of epithelial ovarian cancer. In total, 82 ovarian carcinomas were evaluated. Manual immunohistochemistry was performed on paraffin-embedded human tissue microarrays using polyclonal rabbit primary antibodies: anti-STS. The expression of STS was evaluated using the Immunoreactive Score with two independent observers. Statistical analysis was performed using Pearson´s Chi Squared Test; $p<0.05$ was considered significant. STS expression was positive in 73% of the ovarian tumor samples. When classified by subtype we found STS was expressed in 62% of the serous, 94% of the endometrioid and 90% of the mucinous. These results show that STS expression is higher in comparison to normal ovaries and could therefore play a role in the growth and development of ovarian tumors through the production of active steroid hormones.

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Evaluation of the anticancer effects of quercetin and its fermentation products in human colon cells treated with bisphenol A.

M. en C. Nataly García Gutiérrez*, Dra. Haydé Azeneth Vergara Castañeda, Dra. Ana Gabriela Hernández Puga.
*Autonomous University of Queretaro, ZIP code 76170, Tel: 044 4421139927, nat_gagu5487@hotmail.com.

Bisphenol A (BPA) is a synthetic polymer that is used for the production of packing destinated to the food industry. It's presence has been observed in fluids and tissues of the human body thanks to its ingestion by the capacity it has to migrate from the packing into the food by action of the temperature and pH. Once inside the organism, it is metabolized in the liver and distributed to diverse organs, however, a part returns to the colon to be disposed by the entero- hepatic circulation. Studies show that the BPA acts as a endocrine disruptor in some organs, and several reports have found a possible association between this disruptor and the incidency of several types of cancer as colon cancer, which represents fourth place in cause of death worldwide. On the other hand, it is known that a diet rich in antioxidant compounds as flavonoids and their metabolites produced during the fermentation by the colonic microbiotic, promote the inhibition of transformed cells through the modulation of the expression of implicated genes in biological processes like the arrest of the cellular cycle and apoptosis. Quercetin is a natural flavonoid known for its anticancerous properties, and also for its capacity of catching free radicals as to induce the antioxidant endogenous defense, and regulate the signaling roads through the hormonal receptors. Nonetheless the competitive effect of the quercentin and the BPA have not been studied. That is why, the objective of this research is focused in evaluating the effect of the quercentin and their fermentation products in colon human cells with and without cancer treated by BPA.

Delivery of Resveratrol to cardiac cells through polymeric nanovectors

Paola García Medina[1], Paulina Hernández Fontes[1], Omar Lozano[1,2,*], Gerardo García-Rivas[1,2,+]
[1]Cátedra de Cardiología y Medicina Vascular, Escuela de Medicina y Ciencias de la Salud, Tecnológico de Monterrey.
[2]Centro de Investigación Biomédica, Hospital Zambrano-Hellion, Tecnológico de Monterrey.
* omar.lozano@itesm.mx, + gdejesus@itesm.mx
Dirección: Monterrey, N.L., México 64710. Tél: (81)-8888-0471.

Cardiovascular diseases (CVDs) are the clinical syndromes responsible for the highest death rate worldwide. Amongst them, heart failure is an ending stage of CVDs, where blood pumping is severely reduced by the myocardium in part due to heart remodeling as cardiac myocyte hypertrophy and cardiac fibrosis. It has been shown that Resveratrol, a polyphenol, can reduce hypertrophy in the cardiac myocytes. However, its therapeutic use is limited by low bioavailability and high metabolization rate. To this end, the use of polymeric nanoparticles (NPs) promotes a protected drug transport from such disadvantages, while offering a controlled and sustained release.

The purpose of this work is to understand the new possible interactions between the use of a NP to deliver Resveratrol into cardiac cells, and assess whether this route offers a better way to threat hypertrophy. Resveratrol was encapsulated by poly (D, L-lactic-co-glycolic acid) (PLGA). These NPs were functionalized with either chitosan or polyethylene glycol were used to improve cell internalization and bloodflow circulation time, respectively. The NPs were characterized in terms of size, surface charge, surface functionalization (using Infrared spectroscopy), and kinetics of Resveratrol release. Preliminary results show that PLGA-Resveratrol NPs internalize into cardiac cells, do not cause cytotoxic effects, and can reduce the modulate the hypertrophy in a model of cardiac cells.

Evaluation of the anti-migratory effect of a triple therapy in breast, colon and cervical cancer-derived cell lines

Mical Garcia-Reyes[1], Verónica García-Castillo[1], Alejandro Zentella-Dehesa[2], Nadia J, Jacobo-Herrera[2], Eduardo López-Urrutia[1], Carlos Pérez-Plasencia[1]
[1] FES-Iztacala, UBIMED, National Autonomous University of Mexico, UNAM, Tlalnepantla, Mexico
[2] Biochemistry Unit, National Nutrition Institute of Mexico —Salvador Zubirán", Mexico.

Cancer is one of the most important health problems worldwide. Even when therapies like surgery and radiation can be effective, the worst prognosis is given when there are metastasis signals. Metastasis consists in dissemination of cancer cells through blood circulation; it begins with invasion, when cancer cells lost their adherence to the other cells and extracellular matrix and intravasate into lymphatic or blood circulation and must survive and adhere to vessel cells to extravasate in a new organ. While survival and extravasation are ineffective processes, invasion is very effective, and an important therapeutic target to avoid metastasis.

Previously, our workgroup reported a new pharmacological combination –Doxorubicin, Metformin and Sodium Oxamate– that synergistically targets DNA repair, mTOR pathway and lactate dehydrogenase, and displayed selective cytotoxic effects on cancer cells. These targets are reportedly involved in metastasic pathways too, therefore we assayed the efficiency of the triple therapy in invasion and migration of cancer cells.

We tested the pharmacological combination Doxorubicin, Metformin and sodium oxamate (3F) in breast-, cervical-, and colon cancer-derived cell lines and evaluated its effect in proliferation, migration and invasion. Migration was evaluated by a wound healing assay, while proliferation and cell invasion were evaluated in real time using the xCELLigence® RTCA DP Instrument.

The results showed a decrease in proliferation, migration and invasion within 24 h in most of the cell lines treated with 3F. However, the efficiency was different given that some cell lines were more sensitive to the treatment and presented cytotoxic effects at 12 h posttreatment. Also, we evinced that the effect of the Metformin/Oxamate (Met/Ox) combination had a similar effect, decreasing proliferation, migration and invasion in most cell lines.

Our results proved the anti-migratory and anti-invasivity effect of the 3F and Met/Ox pharmacological combinations; therefore, both are suitable anti-metastasis theraphy cantidates.

Decellularized bovine bone chips as a potential biologic scaffold

Alejandro García-Ruiz, Vanessa Pérez-Silos, Nidia Moncada-Saucedo, Alberto Camacho, Jorge Lara-Arias, Hang Lin, Rocky Tuan, Víctor Peña-Martínez, Lizeth Fuentes-Mera[1,2].
[1] Laboratorio de Medicina Regenerativa. Departamento de Bioquímica de la Facultad de Medicina de la Universidad Autónoma de Nuevo León (UANL).
Madero y Dr. Aguirre Pequeño Col. Mitras Centro sin número. 64460, Monterrey, N.L., México.
[2] +52 (81) 83294173 ext 2588;lizeth46@hotmail.com

Introduction. Decellularization is a promising method to prepare natural matrices for tissue regeneration. Successful decellularization has been reported from various tissues such as skin, tendon, and cartilage. Nevertheless, the process for hard tissues such as bone, has as a challenge, to achieve a successful decellularization but without deteriorating the biological properties of the tissue such as the ability to support cell proliferation and differentiation.

In this study, our objective was to define the optimal experimental parameters to decellularize the natural bone matrix from bovine knee using methods based on the use of H_2O_2 and detergents, even so, preserving the integrity of the matrix.

Methods. Bovine knee bone chips were obtained by drilling; Decellularization was performed using 1 of the following 5 methods.

* 4 cycles of 1 hour incubation with hydrogen peroxide (H_2O_2) at 30% [4C30].
* 1 cycle of 1 h in 30% H_2O_2 [1H30].
* 1 cycle of 1 h in 3% H_2O_2 [1H3].
* 1 cycle of 24hs in H_2O_2 at 30% [24H30].
* Alternating incubation for 4 days with EDTA and 0.5% SDS [Grayson].

The efficiency in decellularization was evaluated in histological sections stained with H & E. To evaluate the ability to sustain proliferation, bone chips were re-cellularized with 150,000 hADMSC/scaffold and viability was measured by the Alamar blue method. The cellularized chips were cultured with osteogenic medium for 14 days and the expression of the maturation markers *RUNX2* and *COLI* was measured by qRT-PCR. Decellularized bone chips mechanical features were also evaluated.

Results. Decellularization efficiency ranging from 60 to 90% was observed between the groups, where 4C30 method proved to be more effective. The integrity of the matrix was well preserved in all the methods, except 24H30. Furthermore, 24H30 showed mechanical properties well below that expected for a hard tissue. All the methods revealed during a 8-day kinetics, a positive proliferation curve; except the 24H30 method whose proliferation rate showed a drastic decrease until day 6. No significant differences were observed in the expression of *COLI* between the groups, in contrast, the expression of *RUNX2* evidenced a significant increase in the cells cultured in the decellularized chips by the 4C30, 1H3, and 1H30 methods.

Conclusion. The 4C30, 1H30, 1H3, and Grayson methods show no significant difference in the proliferation and osteoconduction tests, however, only the 4C30 treatment achieves a decellularization greater than 90%, which allows its use for *in vivo* trials.

Differential expression of proteins in cancer cervical biopsies for the search for new biomarkers

Olga Lilia Garibay Cerdenares[1,2], Berenice Illades Aguiar[1], Sergio Manuel Encarnación Guevara[3], Magdalena Hernández Ortíz[3], Gabriel Martínez Batallar[3], Blanca Guadalupe Gómez Sotelo[1], Luis Antonio Martínez Jiménez[1], Tania Nájera Vázquez[1] and Marco Antonio Leyva Vázquez[1*]

[1]Molecular Biomedicine Laboratory. Faculty of Chemical-Biological Sciences. Autonomous University of Guerrero, Mexico
[2]CONACyT- Autonomous University of Guerrero, Mexico; olgaribayce@conacyt.mx
[3]Proteomics Laboratory, Center of Genomic Sciences-UNAM
*Correspondence: leyvamarco@yahoo.com.mx; Ciudad Universitaria, Ed. C primer piso. Tel.: +52 (747) 4710901

Abstract

Cervical cancer (CC) is the fourth most common cancer in women worldwide with an estimated 528,000 new cases in 2012. The same year in México, had an incidence of 13960 and mortality of 4769 cases. There are several diagnosis methods of CC, among the most frequents are the conventional Pap cytology (Pap), colposcopy and visual inspection with acetic acid (VIA), histopathological examination, tests of imaging and detection of papilloma virus high-risk (HR-HPV) with molecular tests (PCR, hybridization, sequencing). Proteomics is a new tool for the detection of new biomarkers that can be associated with clinical stage, histologic type, prognosis, and/or response to treatment. In this study we analyzed 15 biopsies of CC and were compared with cervical cells collected at the time of sampling. For the proteomic analysis was carried out techniques such as uni and two-dimensional electrophoresis, to identify differential protein profiles PDQuest 7.0 Software and Mass Spectrometry (MALDI-TOF) were used, and *in silico* analysis. FABP4 and PSMD14 expression were validated through immunohistochemistry. The correlation between the expression of FABP4 vs FIGO stage with $p < 0.05$ was determined. The expression of FABP4 could be correlated with the progression of CC.

Chromium Picolinate decreases cortisol levels in *Ovis aries*

Andrea Gómez-Zúñiga[1], Gabriela López[2], Luis Gabriel Contreras-Ferrat[2], José Luis Contreras-Montiel[2], Isabel Guerrero Legarreta[3], Carlos Manuel Romero Ramírez[2], Leticia González-Núñez[2*].

[1]Maestría en Biología de la Reproducción Animal. Universidad Autónoma Metropolitana. Unidad Iztapalapa. San Rafael Atlixco 186, Ciudad de México, C.P. 09340, México, teléfono: 555804-4701.
[2]Departamento de Biología de la Reproducción. Universidad Autónoma Metropolitana Unidad Iztapalapa, San Rafael Atlixco 186, Ciudad de México, C.P. 09340, México, teléfono: 555804-4701.
[3]Departamento de Biotecnología. Universidad Autónoma Metropolitana Unidad Iztapalapa, San Rafael Atlixco 186, Ciudad de México, C.P. 09340, México.
* Corresponding author: gnleti@yahoo.es

Introduction. The nutrition in production animals is required to be balanced. Minerals are necessary in its diet because act in various functions of the body and contribute to the gain of muscle mass. Some researchers have observed that Chromium Picolinate (Pic-Cr) as a dietary supplement contributes to the gain of lean mass, to the decrease in body fat and also modifies cortisol levels in blood.

Objective. Study the effect of Pic-Cr in lambs, analyzing the presence of mRNA of the enzymes involved in the synthesis of glucocorticoids in the adrenal gland, as well as cortisol levels.

Methods. 12 male lambs (*Ovis aries*) of the 4 months old were used, 6 of them were feeded with 10 µg Pic-Cr/kg of weight for 60 days, the other were treated with a normal diet and were used as a control. After of the treatment the lambs were sacrificed and the serum and adrenal glands were obtained. Cortisol was measured in 0.5 ml of serum that was extracted with methylene chloride. Radioimmunoassay was carried out in triplicates using 3H-cortisol (1,2,6,7-3H-hydrocortisone, SA 70.0 Ci/mmol), sheep serum and cortisol for the standard analytical curve. On the other hand, total RNA was extracted from each gland and a Retrotranscription (RT) was performed followed by a Polymerase Chain Reaction (PCR) for the following enzymes: 17α-Hydroxylase/17,20 lyase (CYP17), 3β-Hydroxysteroid dehydrogenase-Δ5 Isomerase (3βHSD), 11β-Hydroxysteroid dehydrogenase type 1 (11βHSD1), 11β-Hydroxysteroid dehydrogenase type 2 (11βHSD2) and Glyceraldehyde 3-Phosphate dehydrogenase (GPDH) which was used as a normalization gene. The PCR products were visualized by the fluorescent dye ethidium bromide. The results were expressed in number of pixels (relative optical density) of the gene of interest with respect to the normalization gene (GDPH).

Results. The cortisol levels of the sheep that consumed Pic-Cr were lower that of the control group ($p<0.05$). The expression of the mRNAs for: CYP17, 3βHSD and 11βHSD2, in lambs that consumed Pic-Cr, did not show any difference with respect to the controls. However, the 11βHSD1-mRNA levels were observed that the decreased considerably with respect to the control group ($p<0.05$).

Conclusion. Pic-Cr inhibits cortisol levels and the expression of 11βHSD1-mRNA that encode an enzyme whose function is to regenerate cortisol. The low concentration of cortisol is an indicator of less stress by to handling or to slaughtering of sheep. These indicators could positively help the profitability and livestock viability.

LPS induce phosphorylation of RACK1 in C6/36 HT cells from *Aedes albopictus.*

Hernández-Hernández FC[2], Cázares-Raga FE[2], Cortés-Martínez L[2], Del Angel-Nuñez de Cáceres RM2, Medina-Ramírez JF[2], Urióstegui-Acosta M[3], Muñoz-Barrios S[3], González-Calixto C[1*]e-mail: cgonzalez@uagro.mx
[1]Facultad de Enfermería No.2, UAGro; [2]Depto. Infectómica y Patogénesis Molecular, CINVESTAV-IPN; [3]Escuela Superior de Ciencias Naturales, UAGro.

Introduction. Diseases transmitted by mosquitoes, as dengue, Chikungunya and yellow fever are important public health problems, insects acquire the pathogen during blood feeding on infected individuals and transmission happens when infective mosquitoes bite a new human host (1). On the other hand, mosquitoes interact with potentially harmful stimuli from the environment, including pathogens as arbovirus, parasites, fungi and bacteria; and chemicals, and to manage these external insults, insects activate detoxication mechanisms and innate immune responses (2, 3). Lipopolysaccharide (LPS) is a component of the outer membrane of Gram-negative bacteria that during infections initiate a strong immune response (4). In mosquito LPS activate mainly the humoral immunity, regulated by two major inducible routes, the Toll and immune deficiency (IMD) pathways, which activate, via signal transduction cascades, several effector mechanisms including the production of antimicrobial peptides (AMPs) and the production of melanin and reactive oxygen species (3). In a previous work we identified in *Aedes albopictus* derived C6/36 cells the signaling *scaffold* protein *Aea*lRACK1, ortholog of a conserved protein in all eukaryotes, with multiple physiological functions as adaptor protein, regulating cellular events including translation and pathway signaling, that coordinates a variety of important cell activities in immune and stress responses is modified under nutrition stress conditions (5).

Objective. In order to analyze the *Aeal*RACK1 behavior during *Ae. albopictus* immune response, in this work we studied the changes in protein abundance and phosphorylation of this protein in C6/36 HT cell line, derived from embryos of the vector mosquito, challenged with LPS.

Materials and methods. Cells were treated with LPS, total proteins were extracted, resolved by two-dimensional electrophoresis and gels stained with Coomassie blue to observe total proteins and phosphoproteins were detected by western blot using specific anti-phosphotyrosine, anti-phosphoserine or anti-phosphothreonine antibodies. Selected protein, displaying major changes in phosphorylation was recovered and characterized.

Results. The protein, confirmed as *Aeal*RACK1 by MS and anti-RACK antibody western blot, changed abundance and phosphorylation levels in the presence of LPS.

Discussion. LPS in mammals have multiple effects on stress-response and immunity functions. Here is showed that LPS has physiological effects on insect cells inducing the phosphorylation of *Aea*lRACK1 and probably activating intracellular signaling. RACK1 proteins are main interacting partner of many proteins as kinases and phosphatases and is involved in translation activities. We suggest that *Aeal*RACK1 can participle on immune response mosquitoes.

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Effect of cocoa intake on the induction of in vitro insulin polymers in obese patients

José Arnold González Garrido, José Ángel González Díaz, Blanca Estela Trejo Sánchez, Diana Cristel Jerónimo Contreras, Carlos Javier López Victorio, Adelma Escobar Ramírez.
Centro de Investigación de Ciencia y Tecnología Aplicada de Tabasco. Universidad Juárez Autónoma de Tabasco, División Académica de Ciencias Básicas.
Carretera Cunduacán-Jalpa KM. 1 Col. La Esmeralda CP. 86690.Tel. (993) 3581500 Ext. 6702, 3581573 y (914) 336 09 28. Cunduacán, Tabasco, México. anorld.gonzalez@ujat.mx.

Introduction. Obesity is a chronic noncommunicable disease caused by an increase in adipose tissue, associated with Inflammation and increase of reactive oxygen species. Obesity leads to an imbalance of antioxidant oxidizing molecules producing a state of oxidative stress. The oxidative stress it produces damage at the level of lipids, DNA and proteins. The insulin protein, it has been described that in a state of oxidative stress is generate chemical changes producing a polymer and decreases your hypoglycemia activity. The presence of this polymer suggests its participation in the initial events to the insulin resistance that occurs in obesity. Recently, the benefit of the consumption of foods rich in flavonoids by their antioxidant activity has been described. Cocoa presents many flavonoids, which present cardiovascular benefits.

Objective. In this work we evaluate the effect of cocoa consumption Rich in flavonoids about formation of insulin polymers and oxidative damage in youth obese.

Materials and methods. A case-control study was conducted. Prior informed consent signature was formed two study groups based on the body mass index (BMI): normal weight (n = 15) and obese (n = 15). Was determined parameters anthropometric, biochemical, hematologic, markers of oxidative damage of lipoperoxidation (MDA, 4-HNE), markers of damage to proteins (carbonyl groups) and antioxidant defenses (SH groups and activity of glutathione peroxidase) and the detection of invitro insulin polymers by electrophoresis Non-SDS. An intervention was performed with the consumption of cocoa for seven days and the previously described studies were repeated.

Results. Changes were observed significant in the markers oxidative damage and the detection of the polymer is observed with decrease after cocoa consumption.

Conclusion. Cocoa consumption reduces the amount of insulin polymers in obese patients, which is associated with decreased oxidative damage. These results suggest the consumption of cocoa as an adjunct in the treatment and prevention of insulin resistance in obese patients.

Restart of the estrous cycle by the estrogenic activity of the mesquite pod extract in ovariectomized rats.

Leticia González-Nuñez[1*a], Andrea Gómez-Zúñiga[2], Socorro I. Retana-Márquez[1], Luis Gabriel Contreras-Ferrat[1], José Luis Contreras-Montiel[1], Edith Arenas-Ríos[1], Yolanda López-Ramírez[1], Jessica Hernández Lázaro[3], Pablo Arturo Salame-Mendez[1].
[1]Departamento de Biología de la Reproducción, Universidad Autónoma Metropolitana, Unidad Iztapalapa. San Rafael Atlixco 186, Ciudad de México, C.P. 09340, México. Teléfono: 5804-4701.
[2]Maestria en Biología de la Reproducción Animal. Universidad Autónoma Metropolitana, Unidad Iztapalapa.
[3]Licenciatura en Biología Experimental, Universidad Autónoma Metropolitana, Unidad Iztapalapa.
*Autor de correspondencia: gnleti@yahoo.es

Introduction. Mesquite (*Prosopis sp.*) is widespread legume, widely used to feed several livestock species and as food source for human populations in several countries. Mesquite contains several phytoestrogens which might have potential estrogenic effects. However, to our knowledge, there are no reports on its possible estrogenic activity or the possible harmful effects on reproductive function.

Objective. Thus, the aim of this study was to evaluate the estrogenic potency of mesquite pod extract (EVM) by its capability to induce changes on estrous cyclicity of castrated female rats.

Methods. Adult female Wistar rats (body weight 250-300 g) were ovariectomized (OVX) and kept under a 14 h light: 10 h darkness cycle with free access to water and food. OVX animals were used after two months of recovery (5 months of age), it is a sufficient period to ensure that ovarian hormone levels are completely exhausted. Groups of 6 rats were daily treated with 0.4 mL propyleneglycol (vehicle: Vh) containing 1.2 g alone EVM or combined with 0.5 mg ICI 182,780 anti-estrogen (ICI). The treatment was administrated by 15 days. Other groups were treated with 12 µg/day of alone estradiol (E2) or combined with 0.5 mg of ICI. Vh or ICI groups were used as experimental controls. Estrous cycles were monitored by daily evaluation of vaginal smears which were stained with hematoxilin–eosin and evaluated with an optic microscope. Vaginal smears were obtained 2 h after the onset of the dark period, under red light.

Results. These results suggest that EVM could have estrogenic activity. When we analyzed the estrus cycle of the OVX rats treated with the EVM hydrophobic phase, it was capable to restart the phases of the estrous cycle, although not in the natural sequence of Diestro, Proestro, Estro and Metaestro. The EVM stimulated the transition frequencies of the phase from Diestro to Metaestro at day 5 of treatment in OVX rat ($p<0.05$). In addition, a greater frequency of Estro was observed at day 8 compared to rats treated with the Vh ($p<0.05$), which were kept in Diestro during the days of treatment. When the EVM was supplied with ICI, we observed a significant difference when compared to the EVM-only group ($p<0.05$), this suggests that the ICI blocked the EVM effect, keeping the OVX rats in the Diestro phase.

Conclusion. The data contribute to a better understanding of the EVM mechanism of action and suggest a slight estrogenic effect on the estrous cycle resumption in OVX rats, which was blocked by an anti-estrogen.

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Expression of LAMC1 and ITGβ1 in cervical-cancer-derived cell lines

Diana Laura Gonzalez-Tolentino[1], Miguel Ángel Mendoza-Catalán[1], Ma. Isabel Zubillaga-Guerrero[2], Torres-Rojas Francisco Israel[1], Ana Elvira Zacapala-Gómez[1]
[1]Laboratorio de biomedicina molecular and 2Laboratorio de citopatología e inmunohistoquímica, Facultad de Ciencias Químico Biólogo Parasitólogo, Universidad Autónoma de Guerrero, Chilpancingo, Guerrero, CP.39087. Tel: 4710901, *(zak_ana@yahoo.com.mx).

Introduction: Adhesion is one of the first deregulated cellular processes in cancer and the molecules involved in adhesion can alter its expression to favor the development of cáncer(1). In several types of cancer has been observed up-expression of LAMC1 and ITGB1(2-3), however, in cervical cancer there are no studies; considering the association of cervical cancer with HPV-AR infection and the mechanisms used by HPV to induce cervical cancer, it is important to analyze the level of expression of LAMC1 and ITGB1 in cell lines of cervical cancer.

Objetive: To compare the level of expression of LAMC1 and ITGβ1 in cervical cells (C33A, HeLa, SiHa) with respect to the non-tumor cell line without HPV infection (HaCaT).

Methodology: The expression of LAMC1 and ITGB1 in the HeLa, SiHa, C33A and HaCaT cell lines was determined by Immunocytochemistry and Western blot, and the expression level of the cervical cancer cell lines (HeLa, SiHa and C33A) were compared with the immortal epithelial cell line (HaCaT).

Results: An up-expression of LAMC1 and ITGβ1 was observed in the HeLa (HPV 18) and SiHa (HPV 16) cell lines compared to the non-tumoral HaCaT cell line. HeLa cells showed higher expression of proteins compared to SiHa.

Conclusions: Infection with the specific genotype of HPV-AR could be a factor involved in the up-expression of LAMC1 and ITGB1. Up-expression of LAMC1 and ITGB1 could be involved in the high capacity to migrate and invade HeLa cells. These results are the basis for subsequent studies to analyze the role of the infection of the different HPV-AR in the alteration of expression of LAMC1 and ITGB1 and their relationship with the development of cervical cancer.

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Respiratory Tract Microbiota in Patients with Acute Respiratory Infections

Guerrero Corona Diego, Instituto Nacional de Medicina Genómica (INMEGEN),

Main Tutor: Dr. Xavier Soberón Mainero (xsoberon@inmegen.gob.mx) Supervising Tutor:
Mirelle Vanessa González Covarrubias(vgonzalez@inmegen.gob.mx)
INMEGEN, Laboratorio de Farmacogenómica, 555350.1900 ext. 1212
Periférico Sur No. 4809,
Col. Arenal Tepepan, Delegación Tlalpan.
C.P. 14610 México, Ciudad de México

Respiratory infections are the main causes of medical consultation in the world. According to the DALYs (Disability Adjusted Life Years) reported by the WHO (World Health Organization), infections of the lower respiratory tract occupy the second position with more than 1,939 DALYs per 100,000 inhabitants, only below the ischemic heart diseases.

Currently, the identification of the etiological agent of the respiratory condition is usually a delayed process of several weeks, which requires very specific microbiological culture techniques, highly trained and specialized personnel and complementarity of the results with antibiotic susceptibility tests and other biochemical test or limited molecular identification protocols.

This research work seeks to characterize the microbiota of the respiratory tract directly in the clinical sample, of infected Mexican patients, through the use of NGS (Next Generation Sequencing) technologies, including: a descriptive study of a group of 8 patients with acute respiratory infections (pneumonia , either community associated or of nosocomial origin) of the lower respiratory tract, from which direct samples (sputum) were taken to perform whole nucleic acid extraction of nucleic acids from the present microbiota. Amplification 16S rDNA (V3-V4) was carried out and subsequently sequenced using Miseq, Illumina® sequencing plataform.Data were processed using the QIIME2 software for taxonomic identification of bacteria and subsequently interpreted in the context of their correlation with clinical history information.

Effect of high fat diet and pirfenidone on gene expression involved in a mouse model of cardiomyopathy

Jorge Gutiérrez-Cuevas[1], Ana Sandoval-Rodríguez[1], Jesús García-Bañuelos[1], Adriana Salazar-Montes[1], José Vera-Cruz[1], Alejandra Meza-Rios[1], Juan Armendáriz-Borunda[1,2]
[1]Instituto de Biología Molecular en Medicina y Terapia Génica, CUCS, Universidad de Guadalajara.
[2]Tecnológico de Monterrey, Campus Guadalajara.
Sierra Mojada 950, Edificio "Q", Tercer Piso, Col. Independencia, C.P. 44340, Guadalajara, Jal.
Teléfono: 10585200, Ext. 33881; correo electrónico: gutierrezcj05@gmail.com

Obesity is a public health concern continuously growing and affecting more than half a billion people worldwide. In Mexican population the combined prevalence of overweight and obesity in adults 20 years old and above was 72% in 2016. Main complications from obesity such as diabetes and cardiovascular disease (CVD) are common; CVD is a leading cause of morbidity and mortality in the world. Approximately 17.7 million people died in 2015, and every 4 seconds someone experiences an acute myocardial infarction; this disease is characterized by free fatty acids (FFA) accumulation, although FFA is the main heart source energy, its excess favors lipotoxicity, which in turn results in cardiomyopathy. Combined high fat diet (HFD) with high sugar (Western diet) induce pathologies related to hyperglycemia, hypercholesterolemia and chronic low-grade inflammation on liver and adipose tissue, including heart tissue with oxidative stress, mitochondrial dysfunction, endoplasmic reticulum stress, as well as cardiac fibrosis in mouse models. Pirfenidone (PFD) has been shown to inhibits the progression of fibrosis in vivo in a variety of animal models and different tissues such as heart. However, apart from inhibiting cardiac fibrosis, little is known about the mechanism of action of this drug on diseased heart tissue. The aim was to analyze the heart of C57BL/6J male mice (n = 4 mice/group) fed with HFD (60% fat, and 55% fructose with 45% sucrose in water) and normal diet (ND) with 18% fat as control. After eight weeks, HFD mice were intervened with switch diet (SD) and PFD treatment (380 mg/kg food) up to week sixteen. Results indicates that body weights of animals fed HFD were heavier than ND mice ($P \leq 0.05$); heart weigh of HFD + PFD increased compared to ND ($P \leq 0.01$) and HFD ($P \leq 0.05$) animals; body weight of SD and SD + PFD decreased compared to HFD mice ($P \leq 0.05$); meanwhile, heart weight of SD and SD + PFD decreased compared HFD + PFD ($P \leq 0.01$). Plasma glucose was higher in HFD than SD mice and insulin tolerance test (ITT) indicates that HFD mice group was significantly insulin resistant ($P \leq 0.05$), while PFD decrease it. Histological results showed by Oil Red O and trichrome Masson's staining that heart FFA and fibrosis were significantly increased gradually until 16 weeks in HFD group; PFD and SD ($P \leq 0.05$) decreased FFA, and fibrosis area was diminished with PFD ($P \leq 0.01$). Harris hematoxylin and hematoxylin and eosin showed inflammatory foci in heart tissue of HFD mice. RT-qPCR results indicate that PFD and SD decreases Srebp1 ($P \leq 0.05$) of HFD mice, a lipid metabolism gene; interestingly, IL6 mRNA levels were down regulated in HFD and SD compared with ND mice ($P \leq 0.05$), and TNF-α mRNA expression with increased in HFD and decreased tendency in HFD + PFD. mRNA levels of oxidative stress genes such as Sod1 and Birc2 (Nrf2) were decreased in SD group, and Birc2 mRNA levels overexpressed in HFD mice compared to ND. Results of this work suggest the cardioprotective effects of pirfenidone.

Pharmacological Evaluation of Aqueous Extracts Of Cancerina (*Semialarium mexicanum)* as a Antiteratogenic And Cytotoxic Therapy

Mario Javier Gutiérrez Fernández [1], Leticia Arguello Correa[1], César Adrián Gómez Correa[1].Ingeniería de Química, Área Biotecnología de la Universidad Tecnológica de Morelia[1]. Av. Vicepresidente Pino Suarez 750, 4ta Etapa Ciudad Industrial, 58200 Michoacán de Ocampo,México. Tel (443) 113-59-00. mgutierrez@utmorelia.edu.mx

Summary: One of the medicinal plants used in our country (Central and South Mexico) is the cancerina (*Semialarium mexicanum).* Traditionally used in the treatment of diseases like ulcers stomach, kidney diseases, diseases of the skin, amenorrhea, uterine infections, diarrhea, vomiting, and psoriasis and other parasites of man. Among the compounds identified in extracts of cancerina are several triterpenoids, sterols and some alkaloids; However, it has not reported on the activity of lectins and records on its effect in epidermoid carcinomas are scarce. Performed a qualitative systematic review in which the bibliographic search was conducted on the bases of national and international electronic publications such as: Scientific American, Medline (PubMed), Scielo (Scientific Electronic Library Online), Cochrane Library, Science Direct, Redalyc (network of scientific journals of Latin America and the Caribbean, Spain and Portugal), as well as the databases of the UNAM and IPN. Using the following keywords: cancerina and *Semialarium mexicanum*. Selected articles under the following criteria: type of publication (revision, clinical studies, trials, meta-analysis), in language (English, Spanish), as well as if the publication makes mention of medicinal plants in traditional Herbalism Latin American. Various applications of aqueous extracts of the cancerina (*Semialarium mexicanum*) were identified and their pharmacological action as antiproliferative therapy (Cancer treatment) were identified. The antiproliferative effect of cancerina (*Semialarium mexicanum*) has been reported in studies in cell cultures of the pulmonary adenocarcinoma (A459), generating apoptosis to middle of the aqueous extract concentrations, however, found that such effects they may be associated with reports of having a generalized cytotoxic effect. A549 results with extracts to 20% reports 68% of apoptosis and C33a 28%. Data regarding culture of HEPG2 cell lines (Carcinoma hepatocellular), RAMOS (Lymphoma Burkitt.) and Culture Control line MC3T3-E1 (preosteoblast of mice), (data in progress). We have standardized the volume and concentration of extract cancerina, which integrate into the final version of the research, as well as outcomes on cancer cell lines concerned. Somehow it is regardless to this experimental data to make the differentiation of this antiproliferative effect or a generalized cytotoxic effect, or both.

Soluble Toll like receptor 2 is augmented in saliva of patients with squamous cell carcinoma.

Rebeca Guzmán Medrano; Silvia Isabel Medrano Rodríguez Facultad de Odontología, Universidad Autónoma de Chihuahua Cto. Universitario s/No.,Cd. Universitaria, Campus 1, Chihuahua, Chihuahua, México. C. P. 31000. Tel. (614)-(1242602). Correo Electrónico: rguzman@uach.mx

BACKGROUND: Squamous cell carcinoma is the most frequent cancer of the head and neck region. In this location, this neoplasm comprises squamous cell carcinomas of laryngeal, nasopharyngeal and oral structures. Oral squamous cell carcinoma (OSCC) contributes to 12% deaths by cancer worldwide and oral cancer risk assessment and treatment methods with identification of new biomarkers are being emphasized to control the incidence and mortality of this disease. OSCC microenvironment is typically inflamed and pattern recognition receptors such as the toll like receptors (TLR) may contribute to OSCC progression. This has been suggested for transmembrane TLR-2 and its co-receptor, CD14. However, soluble forms of these proteins have been identified in saliva. These salivary proteins could be implicated as modulators of disease process in OSCC.
OBJECTIVE: The aim of this work was to define the potential of salivary soluble TLR-2 and CD14 as modulators and biomarkers of OSCC development.
METHODS: Unstimulated whole saliva was collected from ten OSCC free patients and fifteen patients with OSCC. The concentration of CD14 and TLR-2 was measured with a commercially available enzyme linked immunosorbent assay kit. Data were statically analyzed with the IBM SPSS Statistics versión 17. P values < 0.05 were considered statically significant.
RESULTS: Level of salivary CD14 was equivocal between the OSCC free patients (mean, 1.62 ± 0.53 µg/ml) and the patients wwith OSCC (mean, 1.33 ± 0.54 µg/ml) ($P<0.05$). In contrast, the salivary soluble TLR-2 concentration in patients with OSCC (mean, 27.86 ± 6.91 pg/ml) was significantly higher than that in OSCC free group (mean, 15.26 ± 4.61 pg/ml) ($P=0.0001$).
CONCLUSION: Our findings support that soluble TLR-2 in saliva could be a potential modulator of OSCC development and functions as a biomarker for this oral cancer.

Beneficial effects of exercise and metformin on the body composition of aged female Wistar rats

David Hernández Álvarez [1], Betriz Mena Montes [2] Gibrán, Pedraza Vázquez [1], Stefanie Paola López Cervantes [1], Alfredo Morales Salazar [1], Edith Hernández Cruz [1],Roberto Santín Marquez [1], Pedro Posadas Rodríguez [1], Viridiana Yazmín González Puertos [1], Norma Edith López DiazGuerrero [1] Armando Luna López 2, Mina Konigsberg Fainstein [1].
[1]Laboratorio de Bioenergética y Envejecimiento Celular, Departamento de Ciencias de la Salud, Universidad Autónoma Metropolitana Iztapalapa. [2] Instituto Nacional de Geriatría.
Address: San Rafael Atlixco 186, Iztapalapa, CP 09340, Mexico City
Email: mkf@xanum.uam.mx
Telephones: 58044729

Sarcopenia is a syndrome characterized by a progressive and generalized loss of skeletal muscle mass and power, as well as a poor physical performance, which has been strongly associated with aging. The decrease in muscle mass in healthy people starts at 40 years of age with a loss of approximately 8% of mass per decade, up to 70 years, where the loss increases up to 15% per decade. The sedentary lifestyle is clearly involved in the loss of muscle mass, suggesting that increased physical activity may have protective effects. Recent studies have shown that metformin (MTF) can prevent the damage caused by sedentary lifestyle by increasing the capacity of muscular performance. The objective of this work was to evaluate the effect of moderate exercise along with MTF treatment on body mass composition and strength in Wistar female rats. The body composition was determined by means of dual energy X-ray absorptiometry (DXA) and strength was determined using a dynamometer. The results show that sarcopenia in Wistar female rats begins at 12 months of age where there is a decrease in the proportion of muscle in approximately 18% in the hind legs of sedentary rats. On the other hand, exercise helps maintain the muscular proportion observed from 4 to 20 months of age in hind legs, although MTF does not modify the proportion of muscle in rats. It was observed that exercise associated with MTF treatment increases the proportion of total body bone mass in rats treated with MTF from 12 months to 20 months of age compared with sedentary rats of the same age. The strength assessment study showed that both exercise and MTF treatments increased strength in 22-month-old rats. Conclusions: Moderate exercise prevents the loss of muscle mass in hind legs and increases strength, while MTF treatment increases strength in groups of sedentary and exercised rats.

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Expression of BiP, IRE1, ATF6 and PERK (Unfolded Protein Response mediators) in mechanical and alkali corneal lesion in rat

Erandeni Hernández Esparza, Luz María Bautista Velasco, Tonatiuh Barrios García, Raquel Guerrero Alba, Eduardo Emmanuel Valdez Morales, Sugela Susana Blancas Zugarazo
Departamento de Cirugía, Centro de Ciencias de la Salud, Universidad Autónoma de Aguascalientes
Av. Universidad No. 940, Cd. Universitaria, CP 20131, Aguascalientes, Ags. Edif. 107. Tel. 01 (449) 910 8000, Ext. 8432. E-mail: ssblancas@gmail.com

Unfolded Protein Response (UPR) pathway has an important role in cellular homeostasis. This pathway is associated to unfolding protein accumulation in the Endoplasmic Reticulum in the cell. The UPR regulate the protein production to avoid the protein accumulation. There are three main regulators in the UPR, which are IRE1, ATF6 and PERK, and a three-way master regulator, BiP. Activation of UPR pathway has been associated with many diseases including diabetes and cancer. Particularly in ocular damage, this pathway is activated in pathologies as glaucoma but it is unknown its possible activation in corneal lesions. In this work, we explored the UPR pathway role in a rat model of alkali or mechanical corneal lesions, in the way to explore the sights in human corneal diseases like keratoconus.

A corneal lesion model was established and characterized in Wistar rats. A mechanical injure was provoked by corneal de-epithelization by gentle scraping of the central epithelium. On the another hand, an alkali corneal lesions was provoked with NaOH 1M. Both corneal lesions were 3 mm in diameter. Corneal surface integrity was examined at 0, 24, 48 and 72 h after injury by fluorescein staining and tissue recovery was determined by Hematoxylin-Eosin staining. The expression of UPR regulators (IRE1, ATF6, PERK and BiP) was measured by RT-PCR assays.

The tissue examination showed that alkali lesion induced a rapid (at 0 h) loss of corneal transparency and severe epithelial damage that are recovered at 72 h. This corneal lesion induced inflammatory cells infiltration and loss of collagen fibers arrange in the stroma at 24 h. In the mechanical injure, the re-epithelization process was observed at 48 h and there was a complete recuperation of the corneal surface at 72h. These results suggest that alkali lesion is more aggressive that mechanical de-epithelization of the cornea. When we explored the expression of UPR regulators, our preliminary results showed that alkali lesion induce an increment of BiP and IRE levels while mechanical injure provoked the diminish of BiP and ATF6 levels. These results suggest that UPR pathway could be activated in corneal lesions and this cellular response might be associated to the corneal damage process.

Chemoprotective effect of lactoferrin in Hepatocellular Carcinoma

Hury Viridiana Hernández Galdámez[1*], Samia Fattel Fazenda[1]†, Mario Alejandro Aguilar-Chaparro[1], Jonathan Mendoza-García[1], Teresita del N.J. Flores-Téllez[1], Carolina Piña-Vázquez[1], Mireya de la Garza Amaya[1], Saúl Villa Treviño[1**].
[1]Departamento de Biología Celular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Av. IPN No. 2508 Col. San Pedro Zacatenco, México 14, CP 07360, México, Ciudad de México.
e-mail: *huryviridiana@gmail.com; **svilla@cell.cinvestav.mx

The hepatocelular carcinoma (HCC) is the fourth cause of death by cancer in our country, its high mortality is mainly due to late diagnosis and to low effectiveness of available treatments. For the above-mentioned, currently researchers are looking for compounds with chemoprotector potential to inhibit, decelerate or reverse carcinogenesis. A compound with such capacity is lactoferrin (Lf), this is a protein found in mammals secretions such as milk, and its anti-carcinogenic potential has been demonstrated in studies with different cancers. Therefore the aim of the present work was to study the capacity of the bovine Lf (bLf) as a chemoprotector agent for HCC, at the prevention level and as an antitumor agent. It was determined that a single dose of bLf can decrease the onset of preneoplastic lesions in a model of chemical hepatocarcinogenesis in rats. The suggested mechanism is that bLf inhibits the initiation by carcinogen diethylnitrosamine (DEN), since there was a reduction in DEN necrogenic activity and in DEN derived oxidative stress. Finally it was determined the antitumor activity of bLf on HCC cell lines, as bLf was able to decrease the viability of HepG2 and Huh7 cells. To conclude, taking into account the properties demonstrated in this study and its natural availability, bLf could be an ideal protein to be studied further for HCC prevention and as an adjuvant in HCC treatment.

Transcription level of *socs4* and *socs6 genes* in peripheral blood mononuclear cells of patients with Multiple Sclerosis by real time RT-PCR

Adriana Hernández Reyes[1], Virginia Sedeño Monge[2]
[1]Decanato de Cs. Biológicas, 2Decanato de Cs. de la Salud
Universidad Popular Autónoma de Puebla
21 sur #1103 Barrio de Santiago, C.P. 72410, Puebla, Pue., México
Phone number 52(222)2299400 ext. 7494 e-mail: virginia.sedeno@upaep.mx

Introduction. Multiple sclerosis (MS) is an autoimmune disease, with chronic and degenerative characteristics in the central nervous system (CNS), which is characterized by inflammation and demyelination of axonal tissue. Evidence has been documented that demonstrates the participation of pro-inflammatory cytokines, in the progression of this disease. A cellular pathway commonly used in the signaling induced by cytokines is the JAK-STAT pathway (Janus Cinases-Transducers and Activators of Transcription Signals), it is negatively regulated by different proteins; between these, the family of proteins called suppressors of cytokine signaling (SOCS) is one of the most studied, due to its close relationship with autoimmune diseases and the CNS. Eight members integrate the SOCS family (SOCS1 to SOCS7, and CIS). Specifically socs4 and socs6 proteins exert a negative feedback induced by cytokines as de IL-18, IFN-ɣ, IL-6, IL-23 and others, which participate in the physiophatology of MS.

Objective. In the present work we analyze the transcripts of socs4 and socs6 in peripheral blood mononuclear cells (PBMC) of patients with MS, to later correlate them with the progression of the disease.

Methodology. RNA from PBMC was obtained from a group of MS patients who were treated with Interferon beta (IFN-β) or Glatiramer acetate (AG) and a control group (healthy clinically individuals) using Trizol. socs4 and socs6 transcripts will be quantified through real time RTPCR the by the $2^{-\Delta\Delta CT}$ method.

The preliminary results. Ten RNA samples of both study groups were obtained, the standardization of End-Point RT-PCR to socs4 and socs6, and hprt as housekeeping gene has been done. Currently we are obtaining and analyzing results by real time RTPCR.

Conclusion. The study of the socs4 and socs6 transcripts would allow us to propose them as biomarkers for the early detection of MS, and to elucidate a better overview of the progression clinical of the disease.

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Effect of pyrophosphate thimine on the nerve conduction speed in patients with diabetic polyneuropathy

Itzelly Ibarra-Valdovinos[1], Vania Arleim Rojas-García[1], Ricardo André De Luna-Sánchez[1], Juan Carlos Solis-Sainz[1], Pablo García-Solis[1], Adriana Jheny Rodríguez-Méndez[1], Ana Alicia Sanchéz-Tusie[1], Hebert Luis Hernández-Montiel[1]
Clínica del Sistema Nervioso, de Facultad de Medicina, Departamento de Investigación Biomédica, Universidad Autónoma de Querétaro[1].
Dr. Hebert Luis Hernández Montiel
Universidad Autónoma de Querétaro, Facultad de Medicina
Clavel 200 Col. Prados de la Capilla, Querétaro, México.
C.P. 76170. Tel. (442) 192 12 00 Ext. 6252
hebertlhm@yahoo.com.mx

Introduction. Type 2 Diabetes Mellitus (T2DM2) is one of the most prevalent conditions in Mexican population and presents multiple microvascular complications including diabetic polyneuropathy (DPN). Currently, the clinical management of DPN focuses on proper glucose control and symptoms relief. The present study addresses the therapeutic use of thiamine pyrophosphate (TPP), which belongs to a group of potent therapeutic agents that activate the transketolase enzyme. This enzyme is important in the pathogenesis of T2DM2 because it reduces the activation of metabolic pathways associated with damage due to diabetic microangiopathy. The establishment of new therapies that induce the activation of transketolase could diminish the development of alterations such as DPN. Therefore, the objective of this work was to evaluate the effect of TPP on nerve conduction velocity (NCV) in lower limbs and the antioxidant status of patients with DPN. Material and methods. The present study was a controlled, pre-test/post-test clinical intervention study, which was made with patients that showed lower limb DPN determined by the Michigan Test. Two study groups were assigned according to the time of diagnosis of T2DM2: Group I patients with 1 to 10 years T2DM2 and Group II, from 11 to 20 years. Both groups received treatment with TPP (1 mg/kg weight), intramuscularly once a week, for 3 months. The electrophysiological parameters (latency, duration, amplitude and conduction velocity) of the deep peroneal and sural nerves of both lower limbs were analyzed. In addition, biochemical parameters (glucose, cholesterol, triglycerides, etc.) were determined and the activity of the endogenous antioxidant enzyme superoxide dismutase (SOD) was determined before the start of treatment and 3 months after treatment. Results. At the end of the 3 months of treatment, Group I showed a significant improvement in the NCV in the sural nerve on the right side and for Group II, it improved on the left side. Regarding the peroneal nerve, for Group I, there was also significant improvement in the NCV on the left side and for Group II it was improved on the left side. For the antioxidant status, in Group I, an increase in the activity of the SOD enzyme was found; however, in Group II, this increase in SOD activity was more significant. Conclusions. The results show that treatment with TPP significantly improves the NCV in lower limbs, with better response in patients with shorter time of evolution of T2DM2, also TPP increases the enzymatic activity of SOD, and can act as a protector in the DPN.

Key words: Diabetic polyneuropathy, thiamine pyrophosphate, nerve conduction velocity, superoxide dismutase.

Elevated arginase activity levels in patients with central obesity

Diana Cristel Jeronimo Contreras*, Blanca Estela Trejo Sánchez, Adelma Escobar Ramírez, Carlos Javier López Victorio and José Arnold González Garrido.
The Faculty of Basic Sciences of the Juarez Autonomous University of Tabasco (DACB-UJAT).
e-mail: arnold.gonzalez@ujat.mx phone: 271-128-9645 CP. 86280

Introduction. The World Health Organization (WHO) has defined obesity as a chronic disease characterized by an increase in body fat because of imbalance between energy intake and expenditure, which in turn poses a mayor health threat to obese people. Several studies have revealed that body fat distribution in the thoracoabdominal region (also known as abdominal or central obesity) is related to metabolic diseases, which are caused by increased adipocyte inflammation and hypertrophy, and cardiovascular diseases, which are caused by alterations in endothelial cell function. These processes are linked to cytoplasmic arginase activity, I that is mostly found in liver, vascular tissue and M2 anti-inflammatory macrophages, which are responsible for tissue repair and play a decisive role in obesity-associated insulin resistance.

Objective. The current study assessed serum arginase activity as a marker of inflammation in central and peripheral obesity.

Material and Methodology. An exploratory case study was conducted from January to December in 2017. With prior informed consent, blood samples were taken from freshmen who went to the UJAT University's Clinical Testing Laboratory for a medical checkup. The biochemical and anthropometrical parameters (glucose, cholesterol and triglycerides) were analyzed. The prevalence of obesity was determined in the mass body index (BMI) in accordance to the WHO criteria and central obesity was detected by measuring the abdominal perimeter (>102 cm).

Results. The participants of the case study were males with 18 years old on average. Three study groups were made taking normal weight (n=137), overweight (n=21), obesity (n=54) ranges into consideration. The obesity group was divided into two: central obesity (n=46) and peripheral obesity (n=8). A prevalence of obesity of 25.5 percent and a statistically significant difference with a higher arginase activity was noticed in obesity and overweight groups compared to the control group. In the central obesity group, arginase activity levels were found elevated, compared to peripheral obesity ranges.

Discussion and conclusion. The assessed systemic arginase activity levels were considerably higher in patients with abdominal obesity. Inflammatory processes have been described in cell cultures and animal models that led to the activation of immunological responses through M2 macrophages with cytosine production, like the tumor necrosis factor (TNF)-α, which also involves some reactive oxygen species (ROS), whether in adipocytes or endothelial cells, given to the fact that they're affected in their structure by a hypertrophy caused by excess fat in the cytosol. Presented this way, it's a risk for vascular dysfunction, insulin resistance and hypertension. The Arginase activity is a factor limiting polyamine proliferation and synthesis, causing trouble in protein synthesis. This study reveals arginase activity as a marker of inflammation observed in obesity and which increases in central obesity.

Effect of the acute consumption of polyphenol and a program of physical activity in the treatment of the osteoarthritis in older adults

Everardo Antonio Jiménez Respardo[1], Ivonne María Olivares Corichi[1], Héctor Daniel Hernández Hernández[2], Omar González Gutiérrez[4], Magdalena Guadalupe Rodríguez Santiago[3], Issay Saúl Miranda Díaz[3], Francisco Zamora Rosales[1], José Rubén García Sánchez[1]
[1]Escuela Superior de Medicina del Instituto Politécnico Nacional. [2]Centro de Salud Lázaro Cárdenas III del Instituto de Salud del Estado de México. [3]Instituto Tlaxcalteca de Asistencia Especializada a la Salud (ITAES). [4]Radiología e Imagen de Querétaro S.C.
[1]Plan de San Luis y Díaz Mirón s/n, Col. Santo Tomás C.P. 11340. Ciudad de México. México.
jrgarcias@ipn.mx

The osteoarthritis (OA) is a disease of the joints or junctures where one or more joints undergo degenerative changes that mainly affect the cartilage, and that co-exists with muscle weakness in the elderly. In the OA has been reported the existence of an inflammatory condition and oxidative damage that modify the cartilage and that determine the severity of this pathology. There is evidence that reports the polyphenols consumption with antioxidant effects, anti-inflammatory and of remodeling of cartilage, which could have an impact on the control and OA treatment. Also, it has been described the benefits of a program of exercise with resistance on the OA, which generate improvements in the function, the mobility of the joint, and decrease of the pain.

The aim of this study was to establish the effects of acute consumption of polyphenol (cocoa) during the treatment of OA with a resistance exercise program in older adults. Twenty older adults diagnosed with OA participated in the study. They were divided into two groups, one with physical activity and the second with physical activity and cocoa consumption. The joint damage of the knee was established through a magnetic resonance imaging (MRI). The participants also were evaluated anthropometrically (height, weight, body mass index and waist). In addition, a peripheral blood sample was obtained, and it was determined the activity of the enzyme arginase as an inflammation marker. It was established, their redox state through oxidative damage to lipids (MDA), proteins (carbonyls groups, quinones formation), and it was determined the activity of the antioxidant enzyme GSH-Px and total SH groups. The results showed that both groups decreased lipoperoxidation products (MDA) and the inflammatory process (arginase activity).

However, in the group that consumed cocoa there is an improvement in the antioxidant capacity and the MRI indicated that there was a decrease in the edema in the subchondral bone. We conclude that the combination of cocoa consumption with resistance exercise can be a strategy that contributes to the treatment of OA

Association between insulin resistance and anthropometric, physiologic and metabolic risk factors in scholars from the state of Queretaro

Alan M. Juárez-Ramírez*, Marissa Reyes-Bastidas, Ana G. Hernández-Puga, Ana A. Sánchez-Tusie, Haydé A. Vergara-Castañeda, Rubi Mauricio-Castillo, Andres Valdevit, Francisco Campos-Maldonado, Hebert L. Hernández-Montiel, Pablo García-Solís, Juan C. Solis-S.
Department of Biomedical Research, School of Medicine; Autonomous University of Queretaro, Mexico. (: +52(442)1921200, ext: 6240. -: Clavel 200, Col. Prados de la Capilla, Querétaro, Qro. C.P. 76170; México. carlos.solis@uaq.mx

Background: the prevalence of childhood obesity represents a serious public health problem worldwide due to its progression in developed as well as in developing countries. WHO data mention the number of obese individuals aged 5-19 years multiplied globally by 10, increasing from 11 million in 1975 to 124 million in 2016. ENSANUT 2016 found that the combined prevalence of overweight and obesity in scholars from 5 to 11 years old is 33.2%, with an obesity prevalence of 12.2% for females and 18.6% for males. Obesity represents a major risk factor for the development of different metabolic alterations, such as insulin resistance, dyslipidemias and hypertension; thus increasing the risk of early-onset comorbidities such as diabetes mellitus and/or cardiovascular diseases. Insulin resistance is determined by the HOMA-IR calculation, by using the glucose and insulin circulating levels. However, the possible association between HOMA-IR and other metabolic risk factors has not been completely described in our country.

Aim:to determine the possible association between HOMA-IR and other metabolic risk factors in a population of Mexican scholars.

Materials & methods: in this crosssectional study a total of 357 scholars aged 8-13 years old were included. Anthropometry and blood pressure were measured and blood samples were collected in order to determine glucose, cholesterol, triglycerides, LDL-C, HDL-C, VLDL-C, insulin, TSH and fT4.

Results: the observed global prevalence for low weight, normal weight, overweight and obesity were 5.2%, 54.7%, 18.2% and 21.9%; respectively. By employing contingency tables, significant OR values were obtained when comparing those scholars above the 75th percentile for HOMA-IR with scholar above the 75th percentile for percentage of fat mass, LDL-C, Triglycerides, total cholesterol, systolic and diastolic blood pressure.

Conclusions:overweight and obesity prevalence were significantly higher than the national values. Our results show significant associations between HOMA-IR with the anthropometric, physiological and metabolic risk factors. These results could help to identify and prevent the development of metabolic diseases like diabetes mellitus and hypertension.

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EFFECT OF CURCUMIN ON THE PROTEINS GLYCATION AND PPAR EXPRESSION PROTEIN IN HEART OF MICE FED WITH HIGH FRUCTOSE CONTENT

María Cristina León-García, Cecilia Gabriela Meléndez-Salcido, Ma. Eugenia Garay-Sevilla, Katya Vargas-Ortiz, Joel Ramírez-Emiliano and Victoriano Pérez-Vázquez.
Dpt. of Medical Sciences, University of Guanajuato, 37320-Leon, Guanajuato, Mexico.
E-mail: lega.cristina@hotmail.com and vpvazquez@ugto.mx

The high and constant consumption of fructose in the diet has generated great interest in the metabolic disorders that occur, such as the increase in lipid synthesis, the appearance of diabetes mellitus type 2 (DM2**),** the advanced glycation end-product (AGEs) formation and the cardiovascular diseases. Curcumin, the active ingredient of the *Curcuma longa* plant, is a natural pleiotropic compound that has antioxidant and anti-inflammatory benefits. This study was to determinate the curcumin effect on the protein glycation and PPAR expression proteins in heart of mice fed high fructose content.

Twenty-four (6-week-old C57BL/6) mice were assigned to four groups (n=6) and were treated during 15 weeks. Control group (CT) was feeded with standard diet, curcumin group (C) were treated with 0.75% w/w curcumin mixed in their diet, fructose group (F) were given 30% w/v fructose in water, and fructose plus curcumin group (F+C) were treated with 0.75% w/w curcumin mixed in their diet and 30% w/v fructose in water. At the beginning and end of treatment the body weight of the groups was measured, and then mice were sacrificed for obtain variable biochemical measured in blood with routine techniques. Heart proteins were extracted by a phenolic method and quantified by Bradford method. Finally, the protein glycation and PPAR expression were evaluated by Western Blot analysis.

Fructose induced a significant increase in body weight gain of the mice compared to CT group ($p<0.05$), while treatment with curcumin mitigated this increase induced by fructose in F+C group ($p<0.05$). HDL cholesterol levels were lower in F group than CT group ($p<0.05$). Curcumin reduced the LDL concentration compared with CT group ($p<0.05$). Respect to AGEs, a band with molecular weight (MW) of 62 kDa was detected. Fructose increased the glycation of this protein. Curcumin prevented the protein glycation and increased PPAR expression in mice treated with curcumin and fructose.

This results suggest that curcumin regulates the AGEs formation and expression of related proteins in lipids metabolism such as PPAR in heart mice diet-fed high fructose content, therefore, this active ingredient can be useful in the treatment of cardiovascular diseases.

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Expression of ITGβ1 and LAMC1 in cervical samples from patients with HPV infection, Intraepithelial Squamous Lesion and cervical cáncer.

Alejandro Linares-Martínez [1], Oliver Dircio-Galeana [1], Servando Emilio-Clemente[1], Luz Del Carmen Alarcón-Romero[2], Miguel Ángel Mendoza-Catalán[1], Ana Elvira Zacapala-Gómez[1*].
[1]Laboratorio de biomedicina molecular and [2]Laboratorio de citopatología e inmunohistoquímica, Facultad de Ciencias Químico Biólogo Parasitólogo, Universidad Autónoma de Guerrero, Chilpancingo, Guerrero, CP.39087. Tel: 4710901, *(zak_ana@yahoo.com.mx).

Introduction: Cervical cancer (CC) is associated with HPV infection, worldwide it is the fourth most common cancer in women [(1)]. The diagnosis is realized using Papanicolaou or colposcopy, however, it has several false negatives, therefore, several biomarkers are currently being analyzed that can identify premalignant lesions and CC to be used as prognostic biomarkers [(2)]. ITGβ1 and LAMC1 are proteins that participate in cell-extracellular matrix interaction, their up-expression has been associated with progression in different types of cancer [(3-4)], but in CC there have been no studies.

Objective: To evaluate the expression of ITGβ1 and LAMC1 proteins in cervical samples and their relationship with the degree of Intraepithelial Squamous Lesion and the HPV-AR genotype.

Methodology: 96 samples from patients with normal cervix without infection by HPV, normal cervix with infection by HPV-AR, LEIBG, LEIAG and CC were included. The identification of the expression of ITGβ1 and LAMC1 was realized by immunocytochemistry, genotyping by INNO-LIPA and cytological diagnosis by Papanicolaou (realized by a nationally accredited cytotechnologist).

Results: Moderate/high expression of ITGβ1 and LAMC1 was observed in samples with CC (47% and 76%, respectively) and significant differences in their expression and the LEI grade ($p = 0.01$ and 0.001, respectively). Also, the expression of ITGβ1 and LAMC1 show significant differences in relation to the genotype of HPV-AR ($p = 0.017$ and 0.026, respectively), 76% of the samples with negative expression do not present HPV infection and 20% of the samples with moderate/high immunostaining show HPV 16 infection; the expression of LAMC1 was negative in 48% of the samples without HPV infection and 65% of the samples with HPV 16 infection showed moderate/high immunostaining.

Conclusion: the expression profile of the two proteins evaluated in cervical cytologies, suggest that they could be good immunomarkers of progression in precursor lesions to CC. In addition, the significant difference in the level of expression of ITGβ1 and LAMC1 with respect to the viral genotype could suggest them as markers capable of differentiating between transient and persistent infections.

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Evaluation of the correlation between renal diseases and obesity

Jarumi de los Ángeles López Flores, Guillermo Segura Vázquez, Elvia Scarlett López Gómez, José Arnold González Garrido, Carlos Javier López Victorio. Universidad Juárez Autónoma de Tabasco, Carretera Cunduacán-Jalpa KM. 1 Col. La Esmeralda PC. 86690, 5539965215, Javier.lopez@ujat.mx

Objective: Determine the level of obesity of a university population and correlate the presence of urinary diseases with the place of origin of the sample.

Introduction: Obesity (Ob) is a multifactorial disease favored by inheritance, lifestyle, the type of food that occurs over time, when the calories consumed exceed those consumed, which is favored by the excess in intake caloric and lack of physical activity, which favors the increase in the capacity to accumulate fatty acids and the subsequent increase in the amount of adipose tissue. According to the ENSANUT survey of 2012, in Mexico more than 70% of our population suffers from overweight and Ob, the state of Tabasco occupies the fourth place in the country in prevalence of Ob (1,2,3). Ob increases the risk of suffering from NCDT such as diabetes, heart disease, strokes. Chronic non communicable diseases (CNCD) are a public health problem due to the high cost of their treatment, their multiple associated complications and the high prevalence, among the main NCDTs we can distinguish; to Ob, type 2 diabetes and hypertension, which have been associated as risk factors for the development of Chronic Kidney Disease (CKD)(4), decrease in the glomerular filtration rate (GFR) below 60 ml / min accompanied by structural or functional abnormalities, with implications for health (5).

Methodology: Anthropometric measures; weight, height, determination of body mass index (BMI), abdominal circumference (CA), hip circumference (CC), urinalysis, specialized questionnaires with prior informed consent.

Results: Of a total of 159 participating patients, 76.77% of the participants are male and 16.12% are female, of which 20.74% are obese and 87.5% have type 1 obesity and 12.5% type 2, respectively. 73% of the participants come from the municipalities of tabasco; Center, Comalcalco and Cunduacán.

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Antiproliferative, necrotic and apoptotic activity of the glycoconjugates Diosgenin-2-acetamido-2-deoxy-beta-D-glucopyranoside (MF-10) and Diosgenyl 2-amino-2-deoxy-beta-D-glucopyranoside hydrochloride (MF-11) *In Vitro*

Hugo López Muñoz[1*], Sergio Iván Martínez Mata[1], Ma. Luisa Escobar Sánchez[2], Octavio Daniel Reyes Hernández[1]. Jazmin Ciciolil Hilario Martínez[3], Jesús Sandoval Ramirez[3], Luis Sánchez Sánchez[1*].

1. Laboratorio Biología Molecular del Cáncer, FES Zaragoza, UNAM. Iztapalapa México D.F. 09230. 2. Laboratorio de microscopía electrónica, Facultad de Ciencias, UNAM. Coyoacán, México. D.F. 04510. 3. Facultad de Ciencias Químicas, BUAP, Puebla, Pue. 72570 México.
luisss@unam.mx, hugolm@comunidad.unam.mx

Introduction. Currently, the cancer is an important problem health in the globe. The treatments are not selective and cause strong collateral effect, generating the necessity to search natural alternative compounds with antiproliferative activity, low cytotoxic activity and with apoptotic activity. In this regard, some glycoconjugate compounds with antitumor activity have been reported. In this work, the antiproliferative activity, cytotoxic (necrotic) and apoptotic activity of the glycoconjugate compounds MF-10 and MF-11, diosgenin derivatives was evaluated. **Methodology.** Cell proliferation was evaluated by violet crystal technique and carboxyfluorescein incorporation. Necrotic cell death was determined by LDH enzymatic activity in the supernatants from cell cultures; the apoptotic cell death was evaluated by detection of apoptotic bodies by epifluorescent microscopy and detection of active caspase-3 by flow cytometry. **Results and discussion.** The results show that MF-10 and MF-11 compounds affect the proliferative potential to tumor cells MDA-MB-231 and Sk-Lu-1 in a concentration dependent manner, with a CI_{50} of 25 □g/mL for both cell lines, without inducing necrotic cell death. Besides, the detection of compact nucleus, generation of bodies apoptotic and detection of active caspase-3, suggest that these compounds induce to the tumor cells to an apoptotic cell death. **Conclusion.** The glycoconjugates compounds, derivatives of diosgenina MF-10 and MF-11 affect the proliferative potential of the tumor cell lines MDA-MB-231 and SK-LU-1 by means of apoptotic cell death.

Key words: glycoconjugates, steroid saponin, diosgenin, necrotic, apoptosis, antiproliferative

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Antiproliferative effect of naringenin with de coexposition with bisphenol A in colon cancer cells

Sara Julietta Lozano Herrera, Andrés Cruz Hernández, Ana Gabriela Hernández Puga, Adriana Jheny Rodríguez Méndez, Haydé Azeneth Vergara Castañeda.
Universidad Autónoma de Querétaro, Facultad de Medicina.
Dirección. Cayetano Rubio No.28 col. Ensueño, Querétaro, Querétaro
Cel. 4424714356 e-mail. sarajuliettalo@gmail.com

Bisphenol A (BPA) is used to make polymers of daily use in canning coatings. The BPA is leached incorporating itself in the food to be consumed. Bisphenol A is an estrogen disruptor which binds to estrogen receptors (ER) both alpha and beta, and it has negative effects in breast cancer and prostate cancer since these organs have this type of receptors. Bisphenol by its metabolism can reach the colon, an organ that also has ER; However, there are a few studies on the effect of Bisphenol A in the colon. On the other hand some flavonoids are similarly linked to ER but its effect is usually associated with positive health effects, being antagonistic to estrogen disruptors such as bisphenol A. The naringenin has protective effects against colon cancer but it is unknown if it could have an antagonistic effect to the toxicity caused by bisphenol A in the colon. Therefore, in the present study of the objective, the effect of naringenin on colon cancer cells, which will be exposed to toxic effects of bisphenol A. The hypothesis is that the naringenin has effect antagonist to bisphhenol A in colon cells, the methology is employing HT-29 and SW 480 colon cancer cells coexposed with naringenin and bisphenol A, using methodologies of apoptosis, necrosis and proliferation, hoping to obtain less proliferation in cells treated with naringenin.

(Colon cancer, estrogen receptor, naringenin, bisphenol A)

Molecular interactions between human osteosarcoma biomarkers and cancer treatment drugs

Salvador Augusto Macías-Sánchez[1], Nidia E. Beltrán-Hernández[1], Heriberto Manuel Rivera[1*]

[1]Laboratorio de Tecnología de Proteínas de Membrana y Medicina de Precisión, Facultad de Medicina, Universidad Autónoma del Estado de Morelos, calle Leñeros SN col. Volcanes C.P. 62350 Cuernavaca, Morelos, México *m2mrivera@uaem.mx

Osteosarcoma is the most frequent malignant bone neoplasm and is highly metastatic, 95% of the patients develop metastasis, mainly in lungs. The diagnosis of bone neoplasms is generally made through histological evaluation of a biopsy. Clinical and radiological features are also important in aiding diagnosis and to complete the staging of bone cancer, which affects predominantly children and teenagers between 10-25 years old with an incidence of 8/cases/1,000,000. There are several specific serological or molecular markers for bone neoplasms. Recently, in bone tumors, molecular markers have been used to increase the accuracy of the diagnosis and assist in subtyping bone tumors. Once, the diagnosis is specified, the alternative is the chemotherapy that consists in the mix administration of Cisplatin, Methotrexate, Doxorubicin, Isofosfamide, and Etoposide which increase overall survival to 60-75%. There is a constant effort to characterize specific osteosarcoma biomarkers as molecular targets of the drugs used for its treatment. In our lab, in order to investigate potential biomarkers for diagnosing osteosarcoma, the profile expression data from normal (hFOB1.19) and osteosarcoma (SAOS-2 and SJSA-1) cell lines were analyzed by RNA sequencing (RNA-Seq). We could identify 896 genes commonly expressed in SAOS-2 and SJSA-1 osteosarcoma cell lines and metastasis process were related with genes found. In this work, we analyze the molecular interactions between osteosarcoma biomarkers and cancer treatment drugs. First, of the 896 genes, we could identify 24 structures deposited in Protein Data Bank (PDB) at high resolution and the native conformation using VinaProcess as docking approach we analyzed molecular interactions. Molecular docking is a powerful tool to study protein-ligand interactions as a means to understand the mechanism of action or as an initial point for structure-based ligand optimization.

Effects of *V. meridionale* on insulin resistance and HDL function markers in women with metabolic syndrome: a randomized, placebo-controlled trial

Marín-Echeverri C[1], Blesso C.N[2], Galvis Y[1], Espinosa-Moncada J[1], Fernández ML[2], Aristizabal Juan C[3], Núñez V[1], Ciro G[4], Barona-Acevedo J[1].
[1]Escuela de Microbiología. Universidad de Antioquia; [2]Deparatament of nutritional Sciences. University of Connecticut; [3]Escuela de nutrición, Universidad de Antioquia; [4]Facultad de Ciencias Farmacéuticas y Alimentarias, Universidad de Antioquia

Introduction: Low high-density lipoprotein cholesterol (HDL-c) concentration and HDL particle dysfunction are present in people with metabolic syndrome (MetS), who develop insulin resistance. The protective effects of higher consumption of polyphenol-rich fruits are associated in part to improvements in HDL function. *Vaccinium meridionale* Swartz is a Colombian berry rich in polyphenols; however, there are no published studies about its effects in people with cardiovascular risk factors.

Methodology: Forty women (47.2 ± 9.4 years) with MetS according to the ATP-III definition, were assigned to consume *V. meridionale* or placebo for 30 days in a double-blind study with a crossover design, separated by a 4 wk washout period. During the study, participants kept habitual diet and physical activity records and asked to avoid polyphenol-rich foods. At the end of each period, MetS parameters, including blood lipids and glucose, serum paraoxonase-1 (PON1) arylesterase activity, PON1 lactonase activity, insulin, adiponectin, and resistin were measured. PON1/HDL-c, homeostasis model assessment of insulin resistance (HOMA-IR) index, HOMA-2, and quantitative insulin sensitivity check index (QUICKI) were calculated. Finally, J774 macrophages were labeled with [1.2-3H (N)]-cholesterol to measure HDL cholesterol efflux capacity ex vivo. Changes after consumption of *V. meridionale* compared to placebo were analyzed. Due to the heterogeneous nature of MetS, participants were also stratified by their (median) HDL-C in post hoc analyses (low HDL-c <42.5 mg/dL, n=20; high HDL-c >42.5 mg/dL, n=20). Pearson and Spearman correlations were determined according to data distribution.
Results: 92.5% of women included had low HDL-c (<50 mg/dL). There were no significant differences in insulin resistance or HDL function markers between *V. meridionale* and placebo periods in each group of women (below or above the HDL-c median). However, after *V. meridionale* consumption, we found that changes in HDL-c (*V. meridionale* vs. placebo) had a negative correlation with changes in insulin ($r=-0.584$, $p\leq0.05$) and HOMA-2 ($r= -0.490$, $p\leq0.05$), and a positive correlation with changes in QUICKI index ($r= 0.554$, $p\leq0.05$) in women with HDL-c values below median. In addition, changes in PON1 arylesterase activity/HDL-c had a negative correlation with changes in resistin ($r= -0.462$, $p\leq0.05$) in the same group. Furthermore, in women with HDL-c values above the median, changes in PON-1 arylesterase activity had a negative correlation with changes in insulin ($r= -0.503$, $p\leq0.05$) and HOMA-2 ($r=-0.623$, $p\leq0.05$), and a positive correlation with changes in adiponectin ($r= 0.507$, $p\leq0.05$). Moreover, in this last group, we found a negative correlation between changes in cholesterol efflux and changes in resistin ($r= -0.581$, $p\leq0.05$).

Conclusions: *V. meridionale* consumption did not significantly affect insulin resistance or HDL function markers compared to placebo in women with metabolic syndrome. However, changes in insulin sensitivity with *V. meridionale* consumption were positively associated with HDL function markers

MMP-9 in presence of nicotinamide in breast cancer cells

María Guadalupe Martínez Hernández[1], Araceli Rubí Viedma Rodríguez[1], Margarita Díaz Flores[2], Luis Arturo Baiza Gutman[1]

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[1]Morphology and Function Unity, Faculty of Professional Studies Iztacala, UNAM. [2]Biochemistry Investigation Unit, Specialities Hospital, CMN Siglo XXI, IMSS.Av. De los Barrios No. 1 Tel: 56231261 e-mail: hermargu@unam.mx

Matrix metalloproteinases (MMPs) form part of a family of enzymes that depend on metals, degrade extracellular matrix proteins and are involved in different aspects of tumor progression. The levels of these enzymes are regulated by gene transcription and their activities by activation of zymogens and endogenous inhibitors called tissue inhibitors of MMPs or TIMPs. TIMPs normally inhibit their activity or participate in the activation of latent forms of the enzyme, for example TIMP-2 in the activation of MMP-2. A relation has been established between the expression and activity of MMP-9 and the invasive activity of different kinds of cancer. Our objective was to evaluate the effect that nicotinamide has on the invasion, migration, secretion and expression of MMP-9 in the breast cancer cells MDA-MB231. Nicotinamide was used at concentrations of 0.5, 1, 5, 10, 15, 20, 25 and 30 mM. The growth of cell population was evaluated through MTT trail, secretion of MMP-9 was determined using zymography in polyacrylamide gels copolymerized with gelatin. An invasive trail was realized through matrigel covered transwells. Expression by Western blot and real time RT-PCR. Depending on the dose of nicotinamide there was a decline in the growth of cell population, a decrease in the levels of MMP-9 and its mRNA, also reduced concentration of MMP-9 was observed in the conditioned medium. These changes were associated with visible diminution in invasion and migration of MDA-MB231. In conclusion nicotinamide induces cytotoxicity in the breast cancer cells MDA-MB231 inhibiting the secretion of MMP-9 and producing a decline in the invasive activity of cancer cells.

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IGF / IGFR complex expression in the MDA MB 231 tumor line: role of ERβ activation.

Max Alejandro Maximino Rojas, Eduardo Monjaraz Guzmán, Julieta López Cuevas.
Laboratorio de Neuroendocrinología, Instituto de Fisiología,
Benemérita Universidad Autónoma de Puebla.
Calle 14 sur 6301. Col. San Manuel, Puebla. C.P. 72570 Tel. 2 29 55 00 ext. 7311 Email:
max.mr0911@gmail.com

BACKGROUND

Triple negative breast cancer (TNBC) is an adenocarcinoma with an incidence of 15-20% of all patients with breast cancer; often their biological behavior is usually more aggressive and more likely to develop distant metastases. TNBCs lack clinical expression of estrogen receptor α, progesterone receptor and HER2 (ERα-/PR-/HER2-), but it express estrogen receptor β (ERβ+). To date, the evidence reported has implicated that the activation of IGFR/IGF complex has strong mitogenic effects in breast cancer. Therefore, the aim of the present work is to elucidate whether ERβ activation influences the expression levels IGFR / IGF complex in TNBC cells.

METHODS

We used MDA-MB 231 cells, a TNBC cell line from grade IV adenocarcinoma, which was maintained in an estrogen-free culture medium. We conducted a dose-time trial; where the cells were incubated in the presence of DPN, a specific agonist of ERβ. At the end of the treatment, through the RT-PCR technique, we evaluated the expression of IGF1 and IGF2 mRNA and their respective receptors, as well as the insulin receptor (INSR).

RESULTS

Our results suggest that the stimulation of ERβ with DPN modulates the level of expression of RNAm that codes for IGF1 and IGF2, as well as their respective receptors. Interestingly, we do not found changes on the level of expression of RNA for INSR.

MAGNETIC SIGNALING WITH MODIFIED NANOPARTICLES

Ixaura Celeste Medina-Medina, Rosario Moctezuma-Martiñonand Vanesa Olivares-Illana.
Laboratorio de Interacciones Biomoleculares y Cáncer. Instituto de Física, UASLP
Av. Manuel Nava No. 6 Zona Universitaria, San Luis Potosí. 78290, México.
Phone: 4444277461. ixaura.medina@gmail.com

Magnetics nanoparticles can be coated with specifics ligands that enable them to bind to receptors on the surface of cell. When a magnetic field is applied, it pulls on the particles so that they deliver nanoscale forces at theligand-receptor bond.It has been observed that this mechanical stimulation can activate cellular signaling pathways. The apoptosis or programmed cell deth, is the destruction of the cell within order to control the development and proper functioning of the orgamism. The cancer cells acquire to ability to evade these apoptotic mechanisms, which causes the formation of tumors. In the present study we propouse the use of modified nanoparticles with a truncated form of TRAIL (Tumor Necrosis factor-related apoptosis-inducing ligand) produced in the laboratory, in order to trigger the apoptotic pathway in cancer cells H1299 (lung cancer) as a result of a

miRNA expression profiling of liver cancer cells-derived extracellular vesicles: an approach for hepatocellular carcinoma diagnostic

Francisco Alfredo Molina Pelayo[1], Luis Alberto Vega Vilchis[1], Jesús Monroy Rodríguez[1], Rosendo García Carrillo[1], Mónica Sierra Martínez[3], Enrique Chávez Jiménez[4] and Luis Alberto Castro Sánchez[1,2]*

[1]Centro Universitario de Investigaciones Biomédicas, Universidad de Colima. Avenida 25 de Julio 965, Colonia Villa San Sebastián, Colima Col., México, C.P 28045.
[2]Consejo Nacional de Ciencia y Tecnología (CONACyT).
[3]Hospital Juárez de México.
[4]Instituto de Fisiología Celular, Universidad Nacional Autónoma de México.
*Corresponding author: luis_castro@ucol.mx, Tel. (312)-3161000 Ext. 47559.

Hepatocellular carcinoma (HCC) is the sixth most common neoplasia and the second cause of cancer death worldwide. The HCC research is of great interest in oncology because the percentage of incidence and mortality are comparable. Therefore, early diagnosis of this pathology can significantly improve the clinical prognosis of patients. Several studies have shown that extracellular vesicles (EVs) —which are spherical double membrane proteolipids containing various molecular components— are increased in blood of patients with hepatic carcinoma. In addition, it has been suggested that EVs take part in the progression of HCC through the transfer of their molecular content (proteins and nucleic acids) to different cell types. However, despite of the evidence involving EVs in the development of this neoplasia, there are not enough data available regarding of microRNAs contained in liver tumor cells-derived EVs. Therefore, the present work determined the microRNAs expression profile of the HCC cell lines, as well as, the oncogenic microRNAs contained in the EVs secreted by hepatocarcinoma cells of different tumor stages. Once the expression profiles were obtained, some microRNAs were proposed as possible tumor markers for HCC. The results show that there are 5 microRNAs contained in hepatic tumor cells-derived EVs that could operate as diagnostic markers for HCC. Additionally, we identified 5 specific microRNAs in the EVs liberated by well-differentiated HCC cells and 6 microRNAs in the EVs released from poorly differentiated hepatoma cells that could be considered as potential biomarkers for the tumoral grade detection. Finally, we evaluated these microRNAs in the plasma of HCC patients. Our results show an increase of microRNAs expression levels in the EVs-plasma purified of HCC patients compared with non-tumoral patients. These results contribute to the development of new diagnostic alternatives for hepatocellular carcinoma.

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In vitro evaluation of a new bioactive and biphasic implant with potential application in osteochondral tissue engineering

Nidia Karina Moncada-Saucedo, Alejandro García-Ruiz, Vanessa Pérez-Silos, Alberto Camacho, Jorge Lara-Arias, Iván Marino-Martínez, Viktor Romero-Díaz, Erika Coronado-Cerda, Catalina Cortés-Álvarez, Vladimira Torres-González, Mónica Yáñez-Caballero, Hang Lin, Rocky Tuan, Víctor Peña-Martínez, Lizeth Fuentes-Mera[1,2].
[1] Laboratorio de Medicina Regenerativa. Departamento de Bioquímica de la Facultad de Medicina de la Universidad Autónoma de Nuevo León (UANL).
Fco. I Madero S/N, Col. Mitras Centro. C.P. 64460, Monterrey, N.L., México.
[2] +52 (81) 83294173 ext 2588; lizeth46@hotmail.com

INTRODUCTION. Osteochondral defect (OD) is a concerning issue in orthopedic and include defects both in the articular cartilage and the underlying subchondral bone. Osteochondral tissue engineering (OTE) emerged as a promising alternative strategy for OD regeneration. Developing bioactive bilayer scaffolds for OD regeneration has been considered a desirable strategy in order to perform the rehabilitation and reconstruction of both phases simultaneously. The purpose of this study was develop and evaluate physicochemical properties of a biphasic and bioactive scaffold from silk fibroin (SF), bovine cartilage matrix (BCM) and bovine bone matrix (BBM) using chondrocytes differentiated from human adipose derived stem (hADSC) with potential OTE application.

METHODS: The hADSC were isolated, cultured and immunophenotyped by flow cytometry, cell pluripotency was evaluated by histological stains. The decellularization process of BCM and BBM was performed using physicochemical methods. Biphasic scaffolds (BS) were fabricated by fibroin crystallization and the Young's modulus was measured. After BS cellularization with hADSC, the proliferation was evaluated by Alamar blue assay. In order to evaluate the structure of scaffolds matured for 15 days, histological and immunohistochemical analyzes were performed. Finally, cellularity and microstructure were observed by SEM.

RESULTS: The hADSC at passage 3 exhibited typical stem cells surface markers and the stains were positive for chondrogenic, osteogenic and adipogenic lineages. BCM showed 91.59% of decellularization and, also was moderately preserved; in relation to BBM, no cells were observed. The biphasic scaffold constructed showed mechanical feature similar to native cartilage since Young's modulus was 805.01 kPa. Regarding biological features, it was able to support cell growth. Furthermore, cells after culture in chondrogenic media displayed remarkable positive stain for collagen II and aggrecan. SEM images showed *de novo* matrix formation with abundant fibers around the cells and homogeneous porous structure.

CONCLUSION. The biphasic scaffold shows a mechanical strength (805.01 kPa) similar to native cartilage (400-800 kPa), and not only supports cell growth, but also provide a suitable cellular environment for *de novo* matrix secretion with chondrogenic-like phenotype. Taken together, the data suggest that the biphasic BCM-SF/BBM scaffold could be a good candidate for the purposes of osteochondral tissue engineering.

microRNAs implications in the intracellular calcium dynamics regulation in hepatocellular carcinoma

Jesús Monroy Rodríguez[1], Rosendo García Carrillo[1], Mónica Sierra Martínez[3], Enrique Chávez Jiménez[4], Andrómeda Liñán Rico[1,2], Luis Alberto Castro Sánchez[1,2]*
[1]Centro Universitario de Investigaciones Biomédicas, Universidad de Colima. Avenida 25 de Julio 965, Colonia Villa San Sebastián, Colima, Col., México, C.P. 28045.
[2]Consejo Nacional de Ciencia y Tecnología (CONACyT).
[3]Hospital Juárez de México.
[4]Instituto de Fisiología Celular, Universidad Nacional Autónoma de México.
*Corresponding author: luis_castro@ucol.mx. Tel. (312) 3161000 Ext. 47559.

Hepatocellular carcinoma (HCC) is the second cause of cancer death worldwide, since its incidence rate is comparable with the mortality, because of the difficulty of diagnostic and treatment. Several functions in the hepatocyte are regulated by increase and decrease of intracellular calcium (Ca^{2+}). The intracellular Ca^{2+} dynamics is a complex process regulated by ionic channels, ATPases (pumps) and exchangers which are distributed in plasmatic membrane and intracellular organelles. MicroRNAs (miRNAs) are a group of small non-coding RNAs of ~18-25 nucleotides that mediates post-transcriptional and pre-translational regulation through mRNA degradation and/or translation repression. However, despite of the evidence involving miRNAs in the HCC development, there are not enough data available regarding of microRNAs implications in the expression regulation of structures that intracellular Ca^{2+} dynamics-related in hepatic tumor cells. Most of the HCC tumors are constituted by malignant cells with various differentiation grades, which could influence in the liver cancer progression. Therefore, the present work determined the microRNAs contribution in the intracellular Ca^{2+} dynamics and its biological implications in hepatocarcinoma cells of different tumor stages. Our results show that the mRNA expression levels of elements that intracellular Ca^{2+} dynamics-related are dysregulated in both well-differentiated and poorly differentiated HCC cells. In this regard, the expression levels of endoplasmic reticulum structures calcium dynamics-related are tumoral differentiation-dependent. These results were corroborated in the Diethylnitrosamine (DEN)-induced hepatocarcinogenesis murine model. Furthermore, we determinate the microRNAs expression profile of the hepatoma cell lines and DEN-treated mice by microarrays analysis. The results do not show a relationship with the tumor differentiation degree; however, some miRNAs are dysregulated in both well-differentiated and poorly differentiated liver cancer cells. In addition, the bioinformatic analysis show 6 miRNAs that modulate the intracellular Ca^{2+} dynamics. Finally, the systems biology suggested the miRNAs contribution of pathophysiological functions calcium-related in the liver tumor development.

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Effect of rice bran on oxidative stress in older adults with cognitive impairment

Alejandro Morales Aparicio[1], José Rubén García Sánchez[1], Ana María Balboa Verduzco[2], Héctor Daniel Hernández Hernández[3], Guillermina González Rosendo[4], Javier Villanueva Sánchez[4], Ivonne María Olivares Corichi[1].
[1]ESM, 2CICS Sto. Tomás, 4CEPROBI del INSTITUTO POLITÉCNICO NACIONAL. 3Centro de salud Lázaro Cárdenas III del Instituto de Salud del Estado de México.
[1]Plan de San Luis y Díaz Mirón s/n, Col. Santo Tomás C.P. 11340. Ciudad de México. México.
iolivares@ipn.mx

With the ageing, short-term memory problems are frequent. Although the causes of these problems are diverse, inflammation and oxidative stress explain some of the physiopathological mechanisms of these abnormalities of brain function. In this context, the development of strategies that decrease oxidative stress could be an alternative to prevent or contribute to the treatment of diseases related to brain ageing such as cognitive impairment and dementia.

The aim of this work was to provide to elderly people with cognitive deterioration a functional food (marzipan, made of rice bran) with a high concentration of antioxidants and to determine their capacity to reduce the biomolecules damage by reactive oxygen species (ROS). Twenty-two subject from the Centro de Salud Lázaro Cárdenas III Estado de México (21 women, 1 man) participated in the study. Their age was between 60 and 70 years old. They were evaluated and diagnosed with cognitive impairment by a psychologist from CICS-IPN. Firstly, a blood sample was obtained to determine biomolecule damage by ROS. Lipids damage (malondialdehyde (MDA)), protein damage by the formation of quinones and exposing of carbonyl groups. The concentration of SH groups and activity of the enzyme glutathione peroxidase (GSH-Px) were determined as markers of antioxidant activity. The activity of the enzyme arginase was determined as a marker of inflammation. After these determinations, the subjects were instructed to consume a third of marzipan (approximately 16 mg of vitamin E), from Monday to Friday for 3 months. The marzipan was designed and elaborated in CEPROBI-IPN.

The results showed that there was a decrease in lipoperoxidation products (MDA), an increase in the antioxidant capacity (GSH-Px) and a decrease in inflammation (Arginase), after the consumption of the marzipan. In conclusion, rice bran has an antioxidant effect in older adults with cognitive impairment.

Rhythmic Changes in Oxidative Stress Indicators of Ovarian Tissues in Adult Rats Related to Expression on Dopaminergic Receptor Type 1 (RDA1) and DARPP-32 in Different Steps of Estral Cycle.

José Luis Morán Perales, María Corazón Flores Bautista, Dulce Elena Letras Luna, María de Lourdes Millán Pérez-Peña, Nora Hilda Rosas Murrieta, Nidia Gary Pazos Salazar, Octavio Sánchez García, Wendy Argelia García Suastegui y Anabella Handal Silva. Benemérita Universidad Autónoma de Puebla. Centro de Química y Departamento de Biología y Toxicología de la Reproducción, Instituto de Ciencias. Ciudad Universitaria Edificio IC4, Av. 14 Sur 6301, Col. San Manuel, CP 72570. Tel. (222) 2295500 Exts. 7160 y 7163.
moranperales@yahoo.com.mx.

Ovarian dopamine is associate with production of reactive oxygen species and its control and regulation are indispensable on cyclic ovulation. We were postulate the existence of an ovarian dopaminergic system that participates in synchronic and cyclic Hypothalamus-Adenohypophysis-Ovary axis function and spontaneous ovulation in the rat. Then our propose was study the production of some indicators of oxidative stress in fresh ovaries from four-day adult cyclic rats in different times to long of estral cycle and its relationship with expression of RDA1 and DARPP-32 in the ovarian cortex. We dissected the ovaries at 08:00, 14:00 and 20:00h in each day of estral cycle: diestrus-1, diestrus-2, proestrous or estrus and were processed for extraction and quantification of indicators of oxidative stress: malondialdehyde, 4-hydroxy-alkenels and nitrites. The ovaries of other animals were dissected for RDA-1 and DARPP-32 immunohistochemical analysis. The RDA-1 and DARPP-32 expression was higher in estrus day compared with other days of the estral cycle. We observed variation cyclic and circadian rhythm in lipoperoxidation indicators, with maximal concentrations in diestrus-2 compared with other estral cycle days. Consistently, the lower levels of oxidative stress indicators were observed at 20:00h compared with other hours in all days of estral cycle. These results shown that existence of dynamic changes in the metabolism of ovarian tissues and the possibility of an apparent relationship with ovarian dopamine and its control on cyclic ovulation in the rat, particularly in the transition between luteal and follicular phases of ovarian cycle.

DESIGN AND EVALUATION OF SELECTIVE CELL PENETRATING PEPTIDES IN MAMMALIAN CELLS

Rafael Ubaldo Morán Torres, Susana Castro Obregón & Gabriel del Río Guerra
Instituto de Fisiología Celular. Universidad Nacional Autónoma de México, México.
Address: Cto. Exterior s/n, Ciudad Universitaria, 04510 Ciudad de México.
Tel. +52 55 56225663 e-mail: **gdelrio@ifc.unam.mx**

ABSTRACT

Cell penetrating peptides (CPP) are short peptides that can translocate plasmatic membranes, hence these have been used as drug carriers and as drugs themselves in the treatment of different diseases. The limitation of this powerful technology is that it lacks selectivity, in other words, these peptides can carry drugs to any cell, without distinction. This work proposes a solution for this problem, by using two different strategies: Peptidase-Peptide and Drug-Bait. The first one consists in embedding CPP sequences into non-CPP sequences, where the penetrating activity can only be activated by cleaving off the CPP sequence from the non-CPP sequence; for this work the membrane peptidase used was Neprilisin (NEP). In the other hand, the Drug-Bait strategy consist in a favored internalization of CPP in cells due a bait in their cell membrane, which will attract the CPP to the cell, the bait used for this work was phosphatidylserine (PS). Four fluorogenic peptides were designed for each strategy and are being tested in the mammalian cell line HEK293T by epifluorescence and confocal microscopy.

Study of structure, function, and relationship of platelet´s receptors and membrane bilayers in atherosclerosis

Armando Ocampo del Valle[1], and Heriberto Manuel Rivera[1]
[1]Universidad Autónoma del Estado de Morelos, Facultad de Medicina Calle Leñeros S/N, Colonia Volcanes, 62290. Cuernavaca, Morelos. Teléfono 7773297048. Correo electrónico: m2mrivera@uaem.mx Facebook.com/LTPMmx @ltpm_mx

Cardiovascular diseases are the major cause of death globally, the World Health Organization calculate that 17.7 million people die at 2015, representing 31% of global death, an estimated 7.4 million were due to coronary heart disease and 6.7 million were due to stroke. Atherosclerosis which is a chronic-inflammatory nosological entity from blood vessels is the principal disorder of this group. The main characteristic of this pathology is the lipid deposit on the subendothelium layer from medium and large caliber vessels. The interaction between inflammatories signals, different immune cell types, and lipoproteins allows atherosclerosis injuries, also known as atheroma unstable plaques. The unstable plaques have a big necrotic core and thickness of the layer which covers it. When it is delivery take place thromboembolic events. The interaction platelet-endothelium stimulate the release of platelets factors which promote a pro-inflammatory state; stimulating induction, growing and break of the atheromatous plaque. As consequence, thrombus formation could derive in stroke, myocardial infarction, and heart dysfunction, increasing patient's morbidity and mortality, and make them a target of the development for early diagnostic and preventive actions for atherosclerosis. The principal aim of this study is the evaluation between platelet bilayer and integral membrane proteins meanwhile atherosclerosis development. To make these possible we used CHARM36 force field and LIPIDWRAPPER program to build an evaluated lipid bilayer membrane models from platelet average lipid composition respectively. We used 75 membrane platelets proteins related to atherosclerosis deposited in PDB. We focus on interacts with collagen fibers during platelet activation in different stages of plaque development, such as endothelial dysfunction and plaque rupture. We evaluate the association *in silico* and found that enable the function of the certain glycoprotein to have positive effects on preventing and treating prime and secondary vascular events.

Route of food allergens administration is critical for the sensitization of BALB/c mice.

Noé Ontiveros [1], Jesús Gilberto Arámburo Gálvez [2], Norberto Sotelo Cruz [3], Lilian Karem Flores-Mendoza [1], Francisco Cabrera-Chávez [4].
[1] División de ciencias e Ingeniería. Departamento de Ciencias Químico Biológicas y Agropecuarias, Universidad de Sonora. Navojoa, Sonora, 85880, México.
[2] Departamento de Ciencias Químico Biológicas, Universidad de Sonora. Hermosillo, Sonora, 83000, México.
[3] Departamento de Medicina y Ciencias de la Salud. Universidad de Sonora, Hermosillo, Sonora, 83000, México.
[4] Unidad Académica de Ciencias de la Nutrición y Gastronomía. Universidad Autónoma de Sinaloa. Culiacán, Sinaloa, 80019, México.
E-mail: noe.ontiveros@unison.mx

IgE-mediated food allergy is an adverse food reaction triggered after the exposure to allergenic food proteins in susceptible individuals. The symptoms vary from mild and transient to life-threatening anaphylaxis. Notably, most food allergy cases are associated with a limited group of allergens, but there are no validated animal models to evaluate their sensitizing or allergenic potential. Aim. Our aim was to evaluate the intragastric and intraperitoneal administration of the common food allergens ovalbumin and α-casein to sensitize BALB/c mice. One protocol of intragastric sensitization and two protocols of adjuvant-free intraperitoneal sensitization that differ in time points for sample collection (days 35, 14 and 28 from the beginning of sensitization) were evaluated. Ovalbumin (0.2 mg/200µl) or α-casein (0.2, 2.5 mg/200µl) were administered intragastrically to BALB/c mice (n = 6). The same allergens (ovalbumin: 0.05 mg/200µl or α-casein: 0.025, 0.05 or 0.25 mg/200µl) were administered intraperitoneally to other groups of mice (n = 5-6).

Blood samples were collected from the tail vein at days 35 (intragastric sensitization), 14, and 28 (intraperitoneal sensitization) and the protein-specific IgE antibody responses were evaluated using ELISA. Kruskall-Wallis test followed by Dunn's comparison tests were used for comparison of more than two groups.

Unpaired t-tests were used to compare the difference between two different groups. Two out of 18 mice that underwent the intragastric protocol showed detectable levels of anti-ovalbumin IgE antibodies and 2 out of 12 showed detectable levels of anti-α-casein IgE antibodies. Regarding the 28-day intraperitoneal protocol, 21 out of 23 mice showed detectable levels of either antiovalbumin IgE or anti-α-casein IgE antibodies. The concentration of 0.25 mg of α-casein showed the lowest levels of anti-α-casein IgE antibodies ($p < 0.05$).

Contrary, only 3 out of 18 mice that underwent the 14-day intraperitoneal protocol showed detectable levels of anti-α-casein IgE antibodies. The adjuvant-free intraperitoneal administration of food allergens performs better than the intragastric route to sensitize BALB/c mice. The intraperitoneal administration of food allergens seems to be the method of choice in the search of a validated BALB/c mouse model to evaluate the sensitizing or allergenic potential of food proteins.

Caffeine inhibits migration and invasion in MDA-MB-231 breast cancer cell line.

Mario Israel Oregel Cortez[1], Raúl Díaz Molina[1] and Octavio Galindo Hernández[1*]
[1]Departamento de Bioquímica, Facultad de Medicina, Universidad Autónoma de Baja California, Mexicali, Apartado Postal 21100, Baja California, México. *Corresponding autor: Octavio Galindo Hernández, Departamento de Bioquímica, Facultad de Medicina, UABC, Dr. Humberto Torres Sanginés S/N, Centro Cívico, 21000 Mexicali, B.C.; phone: 686 557 16 22 ext: 122, E-mail: octavio.galindo@uabc.edu.mx.

Abstract

Accumulating evidences have reported that caffeine has anticancer effects at high blood concentrations. (1, 2) However, whether caffeine has anticancer effects on breast cancer cells at low concentration, especially at physiologically applicable concentration (<412 μM) is still not well understood. In this study, MDA-MB-231 breast cancer cell line was used as a model. The cells were incubated with varying concentrations of caffeine (0, 250, 500 and 1000 μM). Migration and invasion abilities were determined by wound healing assay and transwell assay. The molecular changes were detected by western blot. Caffeine induces a decrease in the activation and phosphorylation of the FAK and Src kinases, proteins involved in the modulation of mammary tumor metastasis. In addition, caffeine promotes a decrease in the secretion of MMP-9 to the conditioned medium. Moreover, results showed that caffeine could inhibit the cell migration and invasion significantly at physiologically applicable concentration in vivo. All in all, this observation indicated that caffeine may suppress the progression of breast cancer. In summary, these findings demonstrate, for the first time, that caffeine inhibits the migration and invasion in MDA-MB-231 breast cancer cells. This makes caffeine a potential candidate for treating breast cancer which will be a safer and more effective treatment by giving for a long time at physiologically applicable concentration.

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Effect of curcumin on PPARα and MLYCD expression in liver of high fructose diet-treated mice

Luz A. Ortega-Hernández[1], Diana L. Hernández-Tejada[2], Katya Vargas-Ortiz[1], Maciste H. Macias-Cervantes1, Joel Ramírez-Emiliano1, Victoriano Pérez-Vázquez[1].
Dpto. de Ciencias Médicas[1] and Dpto. de Medicina y Nutrición[2], División de Ciencias de la Salud, Campus León, Universidad de Guanajuato, 20 de enero, 929. Col. Obregón. CP 37320. León, Gto. México. E-mail: la.ortega.hernandez@ugto.mx and vpvazquez@ugto.mx. Tel: +52-477-7143-812; Fax: +52477-7167-623.

ABSTRACT

Intake of food with high carbohydrate and calories content like fructose might modificate the expression of hepatic proteins involved in the lipid metabolism, inducing the development of Metabolic Syndrome (SM). The consumption of polyphenols like to curcumin might prevent the SM, by avoid the changes of proteins expression implicated in the lipid metabolism as peroxisome proliferatoractivated receptor alpha (PPARα) and Malonyl-coenzyme A decarboxylase (MLYCD). The objective was determinate the effect of curcumin on the PPARα and MLYCD expression in liver of high fructose diet-treated mice.

Twelve mice were aleatory assigned into four groups and fed for 15 weeks as follow: the control group (Ctrl, n=3) received standard diet (SD), the curcumin group (Cur, n=3) received SD supplemented with 0.75% curcumin, the fructose group (Fru, n=3) received SD and 30% fructose in water, and the fructose plus curcumin group (Fru+C, n=3) received SD supplemented with 0.75% curcumin and 30% fructose in water. At the end of treatment, the biochemical parameters were measured in blood using routine techniques. Liver proteins were extracted by a phenolic method, following of Western Blot analysis.

HDL cholesterol levels were lower in the Fru group than the Ctrl group ($p<0.05$).
Curcumin reduced the LDL concentration compared with the Ctrl group ($p<0.05$).

In the other hand, the PPARα expression were higher in Fru+C group compared with the Fru group ($p<0.05$). Curcumin improved MLYCD expression in the Fru+C group compared with the Fru group in a non-significant way.

These data suggest that curcumin ameliorate unhealthy effects caused by fructose through to modulate the hepatic proteins expression implicated in lipid metabolism and could avoid the development of metabolic diseases.

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CETPI: a new protein involved in the pathophysiology of sepsis and septic shock

Eréndira Guadalupe Pérez Hernández; Ismael Luna Reyes; Víctor Manuel de la Puente Díaz de León; José Sifuentes Osornio; Jaime Mas Oliva
Instituto de Fisiología Celular, Universidad Nacional Autónoma de México
C.P.: 04510; Tel: 56225584; e-mail: jmas@ifc.unam.mx

Sepsis and septic shock are a common problem encountered in the critical care unit, these pathologies could result from the dysregulated immune host response against various kinds of infections (van der Poll T.,et al.,2017), that according to the international sepsis registry are due to Gram negative (41.4%) and Gram-positive (32.4%) organisms mainly (Bhan C., et al.,2016). During Gram negative sepsis, there is a focus of infection that release locally and into the systemic circulation variable amounts of lipopolysaccharide (LPS), the main immunogenic component of this bacteria (Manocha S., et al. 2002). We discovered a new isoform of the cholesterol ester transfer protein (CETP), named CETPI. Unlike the 24 C-terminal residues present in CETP, CETPI has 18 residues with a high concentration of prolines and positively charged amino acids. This confers to CETPI new structural and functional properties, such as the ability to interact with LPS, confirmed by employing peptides derived from the C-terminal domain. (Alonso AL., et al., 2003; García-González V., et al. 2015)

CETP is member of a family of proteins known as the PLUNC (palate, lung and nasal epithelium clone) (Bingle CD., et al. 2002); members of this protein family also include BPI (bactericidal/permeability-increasing protein) and LBP (LPS-binding protein), two related mammalian proteins involved in LPS binding activity in human plasma. In this work we demonstrated that CETPI is expressed in plasma of healthy volunteers so as in patients with infection, sepsis and septic shock, due to presence of Gram negative bacteria. Also, distinct to healthy volunteer's plasma samples, we detected by Western blot two bands corresponding to 69 and 65 kDa in the patient's samples, suggesting a possible post-translational modification in CETPI. Therefore, we consider that CETPI could have a relevant function in the pathophysiology of sepsis and septic shock. At the same time, we are working in a lentivirus system for CETPI over-expression with aims of get the crystal structure and clarify the structural and functional relationship of this new isoform of CETP.

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Bovine cartilage decellularized matrix improves chondrogenic differentiation and Young's modulus of Silk fibroin scaffolds

Vanessa P r ez-Silos, Nidia Moncada-Saucedo, Alejandro García-Ruiz, Alberto Camacho, Jorge Lara-Arias, Iván Marino-Martínez, Viktor Romero-Díaz, Hang Lin, Rocky Tuan, Víctor Peña-Martínez, Lizeth Fuentes-Mera[1,2].
[1] Laboratorio de Medicina Regenerativa. Departamento de Bioquímica de la Facultad de Medicina de la Universidad Autónoma de Nuevo León (UANL).
Madero y Dr. Aguirre Pequeño Col. Mitras Centro sin número. 64460, Monterrey, N.L., México.
[2] +52 (81) 83294173 ext 2588;lizeth46@hotmail.com

INTRODUCTION: Scaffolds can provide a 3-D structure for cartilage regeneration. Decellularized cartilage extracellular matrix (DECM) could provide a natural microenvironment for chondrogenic differentiation; however, these scaffolds have poor mechanical properties. By other hand, silk fibroin is a biomaterial used to produce cartilage scaffold that has shown good mechanical properties. The combination of these biomaterials could produce a 3-D scaffold that simulates the microenvironment for cartilage regeneration and provides a good load support. The aim of this work was evaluated the ideal ratio of salt:DECM able to produce a suitable chondral scaffold.

METHODS: Salt-leaching method was used to fabricate cartilage scaffolds, composed of fibroin with or without DECM at different ratios. Groups 1 to 4, were produced based on the ratio of salt and DECM (control without DECM, 1:1, 2:1 and 3:1 respectively). Mechanical test of scaffolds was conducted in cell-free scaffolds. On the other hand, human adipose-derived stem cells were seeded into the scaffolds and, chondrogenic differentiation was evaluated by glycosaminoglycans (GAGs) quantification, evaluation of chondrogenic markers and histological and immunohistochemical staining after 28 days of culture.

RESULTS: Young's modulus was calculated on cell-free scaffolds. All groups showed a Young's modulus similar to human cartilage native (450-850 kPa), however, Group 2 was the stronger scaffolds (805.01 kPa). GAGs content of group 2 and 3 was significantly higher compared with the monolayer cell control. The expression of SOX 9 significantly increases in all groups of scaffolds, where group 1 shows the higher expression; while AGN and COL II expression was significantly higher in group 1. Histological and immunohistochemical staining shown an enhanced matrix synthesis in de novo and, higher content of collagen II in group 2, followed by group 3, 4 and 1.

CONCLUSION: The group 2 evidenced the better Young's modulus, GAGs content and higher content of collagen II. The results suggest that a combination of fibroin and DECM enhance chondrogenic differentiation and that a higher content of DECM in fibroin scaffolds improves chondrogenic differentiation and mechanical features.

High HPV prevalence and its effects on pro-inflammatory cytokine expression in semen of Mexican patients.

Elvia Pérez Soto[1,2,] Olivia Medel-Flores[2], Oros-Pantoja Rigoberto[1], Mariano Gómez-Santana[2], Sánchez-Monroy Virginia[1*]
[1] Doctorado en Ciencias de la Salud, Facultad de Medicina, Autónoma del Estado de México, Paseo Tollocan S/N, C.P. 50180, Toluca, Estado de México.
[2] Laboratorio de Biomedicina Molecular I, Programa Institucional de Biomedicina Molecular, ENMyH-Instituto Politécnico Nacional. Guillermo Massieu Helguera No. 239, Fracc, La Escalera, Col Ticomán, México D.F. C.P.07320, Ciudad de México, México.
*Corresponding autor: vickysm17@hotmail.com, Tel. 01555729-6300 Ext.55534.

Persistent infection with Human papillomaviruses (HPV) are the most common sexually transmitted viruses and etiological agents of several cancers, including Benign Prostatic Hyperplasia (HPB) and Prostate Cancer (PCa). Clinical and epidemiological evidence has demonstrated that infections may lead to chronic inflammation by immune cells that release inflammatory mediator as cytokines, which drive neoplastic progression as HPB and PCa. However, the natural history of HPV infections and persistent chronic inflammation is poorly unknown. The aim of this study was to estimate the type-specific prevalence of HPV DNA infection of the semen in 69 asymptomatic Mexican patients (29-48 years old) to establish the relationship between seminal HPV DNA infection and pro-inflammatory cytokine expression. Semen samples were collected, and HPV detection was performed by PCR using universal primers, and viral genotypes were detected using multiplex PCR. Pro-inflammatory cytokines such as IFN-γ, IL-1β, IL-6 and TNF-α in the seminal plasma were determined using ELISA. HPV was detected in semen samples (94.2 %, 65/69) with elevated high prevalence of high risk HR-VPH (79.7 %; 55/69). The most prevalent HPV types were HR-VPH 52 (15.9 %, 11/69), HR-VPH 33 (13 %, 9/69) and co-infection with HR-VPH 52 and 33 (13 %, 9/69) and con-infection with HR-VPH 16, 18, 31, 52 and low LR-VPH 6, 11, that it to say HLR-VPH (14.5 %, 8/69). Levels of all cytokines were not differed significantly between HVP infected men and non-infected men, although the most prevalent global quantification was of IFN-γ (605.44 ±639.14 pg/mL) followed by IL-1β (298.75 ±555.12 pg/mL), IL-6 (121.01 ±240.44 pg/mL) and TNF-α (2.98±16.25 pg/mL), these results are values higher than in other population. Interestingly, prevalence HR-VPH 52 was associated with levels of IL-6 versus non-infected men (166.77±28534 pg/mL VS 28.14±66.10 pg/mL; p=0.011, t student) and **HLR-VPH** increased IL-1 β than in non-infected men (162.55±139.56 pg/mL VS 0.6667±0.9428 pg/mL; p=0.036, t student). In conclusion, there are high HPV prevalence of **HR-HPV** and co-infection with **HLR-VPH**. The association of HR-VPH (VPH 52) with IL-6 and **HLR-VPH** (VPH 16, 18, 31, 52, 6, 11) with IL-1β constitute evidence of inflammatory environment

Polymorphism detection in genes associated to isoniazid metabolism for dosage adjustment in San Luis Potosi

Pettet-Ruiz Guillermo[1], Sánchez-Garza Mireya[1], Reinoso-Reyes Joaquín[2], López-Revilla Rubén[1]
[1] Instituto Potosino de Investigación Científica y Tecnológica, [2] Hospital General del ISSSTE de San Luis Potosí, S.L.P.
Dr. Ruben López Revilla. Camino a La Presa de San José 2055, Lomas 4 sección, 78216 San Luis, S.L.P. Tel: (444) 136-9549 rlopez@ipicyt.edu.mx

Introduction: Isoniazid is a first line antituberculosis drug that can be used as a prophylactic treatment for patients with latent tuberculosis. Nevertheless, it requires extended periods, up to 6 months, to erradicate the infection. This can lead to hepatotoxicity in up to 10% of patients treated. There is substantial evidence that suggest that genetic variants between patients are responsable for the rate in wich INH is metabolized, leading to a variable response. Identifying these variants and linking them to a response phenotype can be used to establish personalized medicine programs to reduce adverse effects.

Objectives: To develop a reliable and low cost method based on nucleotide sequencing, for polymorphism detection in *NAT2, CYP2E1* and *GSTM1* genes wich encode enzymes related to isoniazid (INH) metabolism and estimate the fequency of genoptypes and phenotypes related to an increased risk of developing adverse effects in patients of San Luis Potosi. Creation of genetic based dosage guidelines to optimize INH profilactic treatment in patients with latent tuberculosis to reduce the risk of hepatotoxicity or early therapeutic failure.

Methods: We designed DNA primers and optimized PCR conditions to generate pure amplification products that contain clinically relevant polymorphisms of *NAT2, CYP2E1* and *GSTM1.* Sequencing of said products allowed to identify single nucloetide polymorphisms (SNPs) in *NAT2* and *CYP2E1* and infer genotypes and the related phenotypes. *GSTM1* deletion was determined by the absence of a gene specific product in a dúplex PCR with a β-globin amplifictation control.

Results: *NAT2* acetylator phenotype frequencies infered from relevant SNPs found: slow acetylator 31.3%, intermediate acetylator 49.3% rapid acetylator 19.4%. *CYP2E1* risk associated phenotype frequencies: wild type homozygous 66.9%, heterozygous 32.5%, mutant homozygous 0.6%. *GSTM1* null phenotype frequency: null 44.0%, wild type 56.0%. Based on acetylator phenotypes of *NAT2* we designed a dosage guideline for prophylactic tratment of latent Tb. Of 63 patients trated with the adjusted doses, none of them developed hepatotoxicity after 8 weeks of treatments while maintaining a therapeutic effectivity against Tb.

Conclusions: We developed an alternate genotyping method to TaqMan probes and PCR-RFLP wich is cheaper and based on direct evidence. We also found that approximately 80 % of the patients evaluated could benefit from an INH dose adjustment based on their genetics. The dosage regimes designed and tested seem to be adequate for a broad application and effectively reduced hepatotoxicity in patients treated with INH

CLIC5 subcellular localization and participation in Hepatocellular Carcinoma

Teresita del Niño Jesús Flores-Téllez[1*]; Carolina Piña-Vázquez[1]; Jonathan Mendoza-García[1]; Saúl Villa-Treviño[1**].
[1]Departamento de Biología Celular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional CINVESTAV
Av. Instituto Politécnico Nacional 2508, San Pedro Zacatenco, 07360, Ciudad de México, CDMX Tel. 01 55 5747 3800 Ext. 5577
Teresita del Niño Jesús Flores-Téllez and Carolina Piña-Vázquez contributed equally to this work.
*authors e-mail: tnjflores25093@yahoo.com.mx; caropv@yahoo.com;
**corresponding e-mail: svilla@cell.cinvestav.mx

Hepatocellular carcinoma is the main liver cancer (>90%), which reports a global increasing incidence the next years and poor survival rate when it is diagnosed (30.5% in localized tumor stage, and <5% in metastasis stage). In the omics age, there is a major need for studies looking beyond the change of expression of different proteins, studies exploring deep into these proteins functions, and their participation in the development of the disease. CLIC5 is a protein member of Chloride Intracellular Channel family. This family has been reported in different cell compartments, with wide functions and overexpressed in different tumors. Previously, we showed that CLIC5 is overexpressed in a rat model of HCC, human HCC biopsies and in human HCC cell lines, however many questions about its function had not been solved. In the present work we evaluated and delimitated a specific isoform overexpression of CLIC5 through free access human cancer databases (TCGA, Inserm, etc.) and in human HCC cell lines. We report here for the first time the nuclear localization of CLIC5 in HCC cell lines. Furthermore, when these cells are driven to EMT, the subcellular localization of CLIC5 switches to cytoplasmic, suggesting that the specific cellular localization and function of this protein depend on the context or HCC state. We also described the participation of this protein in key cancer cells capabilities such as: migration, invasion and tumorigenic potential. Finally, all this results allow us to propose that not only the overexpression of CLIC5 could be use as biomarker in tumors, also their cellular localization and function is important HCC

YY1 negatively regulates the XAF1 gene expression.

Miguel Angel Quevedo-Castillo[1,4], Berenice Camacho-Moctezuma[1,4], Guillermo Aquino-Jarquin[3], Jorge Melendez-Zajgla[2], Gustavo Ulises Martinez-Ruiz[1,4]

[1]Dirección de Investigación, Hospital Infantil de México Federico Gomez.
[2]Genomica Funcional de Cáncer, Instituto Nacional de Medicina Genomica.
[3]Laboratorio de Investigation en Genómica, Genética y Bioinformatica, Hospital Infantil de México Federico Gomez, 06720 Mexico City, Mexico.
[4]Division de Investigación, Facultad de Medicina, Universidad Nacional Autonoma de Mexico, 04510, Mexico City, Mexico

Address: 162 Dr. Maquez, Cuauhtemoc, Mexico City, 06720
Phone: 52289917 ext 2368
e-mail: gustavobambam@gmail.com

Abstract

XAF1 is a tumor suppressor gene that is silenced in cancer. Low or absent XAF1 expression is associated with resistance to anti-tumoral therapy and worse clinical outcomes. Re-expression of XAF1 induced by transcriptional reactivation or plasmid- or virus-mediated expression of this gene is enough to inhibit the malignant growth. Since DNA structural alterations are not associated with XAF1 transcriptional silencing, the identification of negative transcriptionally regulators is needed. In prostate cancer, an oncogenic role for both YY1 overexpression and XAF1 silencing has been shown. In order to assess if these events could be associated, we first performed gene-reporter assays in which two putative YY1-binding sites located in the XAF1 promoter were mutated. These assays showed that YY1 exerts a potential inhibitory effect on the XAF1 transcriptional activation. To further explore this, we performed ChIP-qPCR assays using specific YY1 antibodies that showed this protein interacts with the two putative binding sites in the XAF1 promoter in cells lacking XAF1 expression. Interestingly, the association of YY1 with the XAF1 promoter was inhibited by the treatment with a well-known epigenetic modulator, Trichostatin-A, which induces transcriptional reactivation of XAF1 gene. Concordantly, we observed transcriptional reactivation of XAF1 gene after downregulation of YY1 expression by transiently transfection with specific YY1-siRNAs. We further analysed whether HDAC1 is needed for YY1-mediated repressive actions, as previously described for other genes. We observed that XAF1 was over-expressed by transiently transfection of specific siRNAs against HDAC1. Due to YY1 is strongly regulated at post-transductional level, as hoped, no correlation between YY1 and XAF1 mRNA expression levels was detected in data from prostate cancer samples retrieved from TCGA consortium. Thus, an oncogenic arm of YY1 is achieved through its association to the promoter of XAF1 gene silencing it in prostate cancer.

The effect of administrating an extract from pitaya juice rich in betalains on cisplatin induced nephrotoxicity and cellular damage, *in vivo* and *in vitro*

Yadira Ramírez-Rodríguez; Joyce Trujillo-Silva; Karina Robledo-Márquez; Lina Riego-Ruíz.
Instituto Potosino de Investigación Científica y Tecnológica (IPICYT, DBM-CIIDZA) Camino a la Presa San José # 2055 Lomas 4a sección. San Luis Potosí, S.L.P. C.P 78216. Email: yadira.ramirez@ipicyt.edu.mx Tel: (444) 834-2000.Ext. 7312

Oxidative stress results from a misbalance formed by an excessive reactive oxygen species (ROS) and a reduction of anti-oxidative factors, causing damage that usually ends up in the cell's death. Cisplatin (CP), which is an antineoplastic used for solid tumors, frequently causes hepatoxicity, ototoxicity and nephrotoxicity; in part due to generation of ROS, mainly for superoxide anion. It has been corroborated that there are bioactive compounds that aid to counter balance the oxidative effects of CP induced ROS. These antioxidative compounds are the polyphenols, pectins, sterols, flavonoids and betalains that can be naturally found in some fruits like grapes, pear cactus and pitaya; the last one being an understudied Mexican fruit. Thus the objective of this study is to analyze the effect of an extract of pitaya juice rich in betalains on nephrotoxicity and cellular damage induced by cisplatin *in vivo* and *in vitro.* An extract of pitaya juice rich in betalains (JPBE) was obtained, we include a study *in vivo* where we obtained a curve dosage-response using 5 groups of rats. One control group (n=2; V), a second group working with a single dose of cisplatin (n=3; 6mg/Kg; i.p), and a third, fourth and fifth group (n=5) that were administered daily 2, 4 and 8 ml of JPBE in a water dispenser for 10 days previous to the application of CP, and then we continued with the extract administration for 3 more days. Food and water supply, and the weight of the test rats was registered daily, at the end of the experiment the rats were sacrificed to harvest samples of plasma and renal tissue to determine renal function by creatinine and urea levels. A study to evaluate in an *in vitro* model, the effect of cellular damage of CP in *Saccharomyces cerevisiae* yeast. The CP administered did not modify the rat's body weight, but it altered renal function rising the levels of both creatinine and blood urea, which were significantly lower in the group that received 4 ml of JPBE. The same phenomenon was observed in the left kidney mass. Due to the obtained results with the different dosses of JPBE, we can suggest a change in the dosage mode to improve the bioavailability of the antioxidant compounds and have a better protective effect on the kidneys and the yeast. Additionally, in the *Saccharomyces cerevisiae* yeast, it was observed that the exposition to CP accelerates its cellular damage, which is why we pretend to study the effect of JPBE on the cell's death caused by CP.

Polymorphism rs2295490 in the *TRIB3* gene and its relationship with lipid metabolism in patients with type 2 diabetes

Xochilth Natividad Ramos Deloya[1], Dulce Danira Sánchez Diaz, José Ángel Cahua Pablo, Luz del Carmen Alarcón Romero, Amalia Vences Velázquez, Guillermina Vences Velázquez, Eugenia Flores Alfaro*.
[1]Laboratorio de Investigación de Epidemiologia Clínica y Molecular de la Facultad de Ciencias Químico Biológicas. C. P. 39070, teléfono: 01 (747) 4719310. Correo: efloresa_2@hotmail.com

ABSTRACT

Type 2 diabetes (DT2) is a complex disorder that has a heterogeneous environmental and genetic background. Insulin maintains glycemic homeostasis and participates in the metabolism of lipids by suppressing the release of fatty acids and promoting the synthesis of triglycerides in adipose tissue. The Q84R polymorphism (rs2295490) in the *TRIB3* gene plays an important role in the inhibition of the insulin signaling pathway by interacting with AKT. This SNP has been associated with insulin resistance. **Objective.** In this work, the relationship between the Q84R polymorphism in the TRIB3 gene with DT2 and other conditions related to insulin resistance was evaluated. **Methodology.** A genetic association study was carried out between cases (DT2) and controls (without DT2), residents and born in the state of Guerrero, without kinship among them and with ancestry of Guerrero ancestry. Somatometric and biochemical measurements were made, DNA extraction was made from peripheral blood leukocytes by the rapid non-enzymatic technique, and genotyping of the Q84R SNP was performed by real-time PCR using Taqman probes. **Results.** The genotypic frequencies of the SNP rs2295490 in people with

T2D were 84.4% for the AA, 10.8% for the AG and 5% for the GG. In the carriers of the AG genotype, high serum concentrations of total cholesterol were identified, in comparison with the carriers of the AA or GG genotypes ($p = 0.03$), while with the GG genotype there was a slight decrease in the cholesterol concentration of the high density lipoproteins (c-HDL) ($p = 0.04$). **Conclusions**. Our results show a significant relationship between the Q84R polymorphism of the *TRIB3* gene and the lipid metabolism in people from Guerrero with T2D.

Key wperds: *TRIB3*, Lipid metabolism, Insulin, Type 2 diabetes, Akt.

Genetic and Immunologic Biomarkers in Pulmonary Arterial Hypertension

Luisa María Reyes Cortés, Eduardo Vázquez Garza, Carlos Jerjes Sánchez Díaz, Alicia Ramírez Rivera, Víctor Manuel Treviño Alvarado, Gerardo de Jesús García Rivas*
Instituto Tecnológico de Estudios Superiores de Monterrey, Escuela de Medicina y Ciencias de la Salud, Grupo de Enfoque Medicina Cardiovascular y Metabolómica

Pulmonary Arterial Hypertension (PAH) is a progressive disorder involving the obstruction of small pulmonary arteries, resulting in increased pulmonary vascular resistance and pulmonary pressures. PAH is a devastating disease with no cure, despite the current therapeutic innovations. Mutations in one receptor of the transforming growth factor-beta family (BMPR2) has recently been shown to be present in the majority of cases of inherited (familial) pulmonary arterial hypertension (HPAH) while pathogenic variants in other genes (i.e., ACVRL1, KCNK3, CAV1, SMAD9, BMPR1B,) that are considerably less common have been related to the idiopathic form of the disease (IPAH). Inflammation also appears to be a prominent pathologic feature in PAH as suggested by infiltration of inflammatory cells, including macrophages and T and B lymphocytes and changes in the lymphocytic population in the bloodstream have been reported. Since the confirmatory gold standard test is the right heart catheterization, an invasive procedure, a simple non-invasive test is much needed. Search for novel biomarkers that can be detected by a simple test is ongoing and many different options are being studied. The aim of this project is to determine possible genetic and immunophenotypic biomarkers analyzing 21 genes that have been related to the development of the disease as well as the immunologic response in the form of changes in the populations of monocytes, NK cells and T and B lymphocytes.

* Jefe de grupo
Dr. Gerardo García Rivas
Profesor- Investigador
Director del Centro de Investigación Biomédica
Co-Titular de la Cátedra Cardiología y Medicina Vascular
Escuela de Medicina y Ciencias de la Salud
Tecnológico de Monterrey
Contacto: gdejesus@itesm.mx
Tel. +52 (81) 8888-0472

HYPOGLYCEMIC COMPONENTS PRESENT IN *Nasturtium officinale*.

Andrea Rico-Pedraza [1]*, Edgar Vázquez-Contreras [2], Graciela Letechipía-Vallejo [3], Sergio Gutiérrez-Castellanos [4], Aarón Vázquez-Hernández [5] and Bertha Fenton-Navarro [**1]
[1] Glycobiology Laboratory, [3] Neurosciences Laboratory, [4] Molecular Citopatology Laboratory. Postgraduate Studies Division, Faculty of Medicine and Biological Sciences –Dr. Ignacio Chávez", Michoacán University of Saint Nicholas of Hidalgo (*UMSNH*), Morelia, Michoacán, Mexico. [2] Natural Sciences Department, CNI, UAM Cuajimalpa, 5Specialties Hospital, CMN SXXI, IMSS, Mexico City, Mexico.
*qfbandyrico@gmail.com,
** bertha00_mx@yahoo.com

Introduction: There is evidence that there are about 800 plants with antidiabetic potential. Also, more than 200 compounds of vegetal origin have been reported that show a hypoglycemic effect, including phenols, polyphenols, flavonoids, carbohydrates, and peptides are included, which can be feasible alternatives for the control of diabetes and its complications. In our work group, the hypoglycemic property of watercress has been reported. This work is directed to identify the active components in this plant.

Objective: To identify the main hypoglycemic active components present in watercress PWF and to evaluate their hypoglycemic effect in a type 1 diabetes model.

Material and methods: Acetonic and alcoholic extraction, a post-washed fraction (PWF). Molecular exclusion chromatography. Quantification of phenols, polyphenols, and flavonoids. Acute evaluation of hypoglycemic capacity was performed using glucose tolerance curves in type 1 diabetic male Wistar rats (from 200mg) randomly distributed in the following groups (n=6): Healthy control + control compound (gallic acid or quercetin), healthy + PWF , diabetic + saline solution, diabetic + insulin, diabetic + control compound (gallic acid or quercetin) and diabetic + PWF**.**

Results: The presence of phenols and flavonoids was found in a concentration of 168.6 mgEAG/g and 46.4 mgEQ/g respectively. Hypoglycemic reduction was the following: Saline 8%, insulin 72%, gallic acid (phenols) 27%, quercetin (flavonoids) 80 %, and PWF 73%. There was no statistically significant difference between the groups treated with insulin, quercetin, and PWF.

Conclusion: Watercress PWF main active compounds is flavonoids and phenols. Flavonoids presented activity without significant differences with insulin.

Keywords: diabetes, hypoglycemic, active compounds, medicinal plants.

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Mass spectrometry as a tool to detect secretion proteins of mesenchymal stem cells used for cartilage regeneration.

Ríos-Castro Emmanuel1, Sánchez-Hernández Cristian Uriel1, Arroyo Paz Francisco3, Tapia-Ramírez José1,2
1Unidad de Genómica, Proteómica y Metabolómica (UGPM), LaNSE, CINVESTAVIPN, Ciudad de México, México. 2Departamento de Genética y Biología Molecular, CINVESTAV-IPN, Ciudad de México, México. 3SportMed, Clínica Médica, Guadalajara Jal. México.
Contact: eriosc@cinvestav.mx, jtapia@cinvestav.mx 01 (55) 5747 3800 ext. 1746

Mesenchymal stem cells (MSCs) are multipotent adult cells with fibroblastoid morphology and plasticity towards different cell lineages. MSCs can be isolated from bone marrow, umbilical cord blood, adipose tissue, pancreas, liver, skeletal muscle, dermis, synovial membrane and dental pulp. MSCs can carry out chondrogenic and osteogenic processes, and therefore, have been used in cell therapies in patients with articular cartilage deficiencies providing them improvement. Evidences show that the betterment can be due to the proteins secreted by the MSCs.

The aim of this work was the identification of proteins presents in the culture medium of MCSs isolated from adipose tissue. To reach this objective, we used LC-ESIUDMSE mass spectrometry. In this way, Synapt G2-Si was used to perform the spectrometric analysis. A total of 148 secreted proteins were identified and analyzed by STRING and Cytoscape v3.6.1 software to create an interactome. From total identified, 71 proteins are interacting between them and six main nodes of interaction were created. The analysis of the two main nodes revel the interaction of proteins as COL1A2, FN1, TIMP1, HPX, SPARC, THBS1, LGALS3BP, TGF-β1, and NTRK1 involved in remodeling and configuration of the extracellular matrix as well as in the chondrogenic differentiation of MSCs. We concluded that the secretion proteins analyzed could participate in the creation of an adequate niche promoting differentiation of MSCs to repair cartilage. Subsequent analysis to the rest of identified p roteins should be done to fully understand the interactome as well as their molecular participation during the cartilage regeneration.

Risk of Peripheral Arterial Disease and Metabolic Syndrome in adults of the ISSTEP Hospital

[1]Ma. de los Ángeles Rivera Juárez, [1,2]Clara Luz Pérez Quiroga and [3]Fernando José Cruz Lastra.
[1] Research Area, Deanship of Health Sciences, UPAEP, [2] Area of Physiatry, Specialties Hospital May 5, ISSSTEP, 3 Faculty of Medicine, UPAEP.
Correspondence: 21 sur 1103 Barrio de Santiago, C.P. 72410 Puebla, Pue., Mexico. Telephone: +52 (222) 229 9400 ext. 7494. Email: maangeles.rivera@upaep.mx

Introduction: Peripheral arterial disease (PAD) is one of the clinical manifestations of atherosclerosis. It's diagnosis is associated with an increased risk of cardiovascular events (CVE).1 Metabolic syndrome (MS) is a multifactorial disease that progressively deteriorates health and leads to the development of cardiovascular diseases in the adult population.2 Worldwide, CVE caused 15.2 million deaths in 2016.3 The

objective of this project is to analyze if the adults of the ISSTEP hospital with a risk of peripheral arterial disease have alterations in glucose levels, triglycerides, HDL cholesterol, obesity abdominal and blood pressure characteristic of the metabolic syndrome.

Methodology: A Cross-sectional study. Population, patients of both sexes aged 42-62 years attending the Internal Medicine Service of the ISSTEP Hospital. Patients with atherosclerotic PAD were identified by the ankle-brachial index tests (the reference value was 0.9-1.39) and mainly segmental arterial pressures. The abdominal perimeter was also measured. Regarding the diagnosis of the metabolic syndrome, the Harmonized Criteria of the Program Adult Treatment Panel III (ATP III) were followed; the biochemical tests were performed through spectrophotometric tests, using the kits (Enzymatic Glicemia AA, TG Color GPO / PA AA and HDL Cholesterol fast, Wiener lab.).

Results: A total of 36 patients who were diagnosed with atherosclerotic PAD risk and who agreed to participate in the study, through informed consent, make up the study sample. We found that 61.1% of the patients presented a waist circumference ≥88cm and ≥94cm (women and men respectively), 36% presented triglyceride levels ≥150 mg/dL, however, no patient presented low levels of HDL lipoproteins, this represents a benefit for the health of the patients since although they present/display abdominal obesity and high levels of triglycerides their levels of good cholesterol are optimal and this reduces the probability that they develop hypertriglyceridemia. On the other hand, we found that 3 patients (8.3%) had fasting blood glucose ≥ 100 mg/dL and of these one takes medications to control type 2 diabetes. Regarding systolic/diastolic blood pressure we found that 12 patients (33.3%) they presented pressure levels ≥130/85 mm/Hg, of these only 3 have been previously diagnosed with arterial hypertension and consume medications to control it. Following the criteria of ATP III, we found that 11.1% of patients present metabolic syndrome, it is necessary to analyze this phenomenon in a larger sample so that the results can be taken as statistically significant.

Conclusions: 11% of patients were diagnosed with MS, which is why they present a higher risk of developing CVE. The results of this study can help health personnel to make informed decisions about establishing preventive measures for these diseases.

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Celecoxib increases toxicity of several clinical anti-cancer drugs in cervix carcinoma growth

Diana Xochiquetzal Robledo-Cadena and Sara Rodríguez-Enríquez.
Departamento de Bioquímica, Instituto Nacional de Cardiología, Ignacio Chávez. Juan Badiano #1 Col. Sección XVI Del. Tlalpan C.P 14080 Mexico D.F. Phone 5573 2911 ext. 25307.
E-mail: xochiquetzal@ciencias.unam.mx

Background: In the clinic, several anti-cancer drugs as cisplatin, paclitaxel and doxorubicin used as first line treatment against cervix carcinoma promotes severe adverse effects affecting patient's quality life. In order to decrease the known side effects, we are interested in the evaluation of celecoxib (CELECO), a nonsteroidal anti-inflammatory drug (NSAID) that decrease tumor oxidative phosphorylation [1] in combination with well-known antineoplastic drugs to attain a synergistic effect [2].

Results: Clinical antineoplastic drugs cisplatin, doxorubicin and paclitaxel were combined with CELECO, at sub-IC50 doses and tested in the growth of HeLa cervix multicellular tumor spheroids (MCTS), a physiological model that resembles the initial avascular stages of solid tumors. Drugs alone or in combination were added at the beginning of the MCTS growth (day 0, diameter of 100 nm, n=3, preventive therapy) or during MCTS exponential growth (day 10, diameter of 500 nm, n=3, curative therapy). Canonical drugs (cisplatin, placlitaxel, doxorubicin, carboplatin and gemcitabin) revealed a growth spheroid IC50 in micromolar range (40-160 μM) in both preventive and curative therapies. Combination of gemcitabine, carboplatin and cyclophosphamide at sub-IC50 doses with CELECO (sub-IC50 doses: <1nM for preventive, and <10 μM for curative therapies) had an infra-additive effect in which their respective IC50 increased from nanomolar to micromolar range. On the contrary, CELECO (sub-IC50 doses) increased the potency of paclitaxel and doxorubicin, decreasing their IC50 from 16 nM - 86 μM to 5 nM – 10 μM under both assayed protocols. For cisplatin, CELECO had a synergistic effect most effective than for paclitaxel and doxorubicin. CELECO increased cisplatin toxicity decreasing the IC50 from 50 nM - 270 μM to 8 nM - 10 μM.

These results clearly indicate that CELECO may be used in combination with paclitaxel, cisplatin or doxorubicin as a potential combinatory therapy against cervix cancer.

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Transcriptional Expression of the Unfold Protein Response Genes in Corneas from Patients with Keratoconus

Eréndira Rosas-Ginez; Diana Alvarado-Jiménez; Sugela Susana Blancas Zugarazo; Jose Rafael Villafan-Bernal; Luis Fernando Torres Bernal; Tonatiuh Barrios-García; Raquel Guerrero-Alba; Eduardo Emmanuel Valdez Morales.
Universidad Autónoma de Aguascalientes, Departamento de Cirugía, Centro de Ciencias de la salud, Av. Universidad # 940, Ciudad Universitaria, C. P. 20131, Aguascalientes, Ags. México.
qbvaldez@gmail.com

Introduction: The cornea is a transparent tissue located in the anterior part of the eye, as a high refractive structure, one of the most important function is to allow light input inside the ocular globe. Hence, cornea provides structural integrity to the eye and it acts as barrier against pathogens. Keratoconus (KC) is a vision disorder produced when the round cornea becomes cone like shape causing mild or severe blurred vision. The etiology of this disorder remains to be discover. It has been proposed that oxidative stress might be linked with KC development, as shown in several studies where the gene expression with proteins related to oxidative stress in KC are deregulated. Therefore, we are interested in the study transcriptional expression of the unfold protein response (UPR) components.

Aims: we sought to determine the mRNA gene expression of GPR78 (BiP), ERN1 (IRE1), ATF6 and EIF2AK3 (PERK) then stablish if those genes are involved in the physiopathology of KC.

Methods: The mRNA extraction was made with standard method with TRIZOL®reagent, the UPR gene expression was examined by using endpoint RT-PCR. We used the central button of tissue of the human corneas from patients with KC undergoing to transplantation surgery. The mRNA control was obtained from death donors, and because the central button tissue is mandatory to be used for transplantation, we used the periphery of the donor's cornea as control. The tissue was provided kindly from the Aguascalientes Eye Bank C.A.

Results: The relative expression of the UPR components in KC when are compare with control was: GPR78 was significantly 60% less in KC, ATF6 remained unchanged, ERN1 was slightly lower in KC but still not significative and EIF2AK3 was significantly 70% higher in KC.

Conclusion: Interestingly we found GPR78 (BiP) mRNA at low levels in KC suggesting the lack of the natural inhibitor of the function of the UPR signal pathway. On the other hand, we found a high level of expression of the EIF2AK3 (PERK) suggesting that probably the machinery of protein translation might be affected. We are expecting to do more experiments and see if ATF6 remain unchanged and if there is significance with ERN1 (IRE1) in KC compared with control.

Molecular iodine/all trans retinoic acid as an effective neuroblastoma treatment

Bertha Rueda Zarazúa, Gabriel Rodríguez Gómez, Bertha Lizeth Godoy, Diana Organista Juárez, Evangelina Delgado González, Brenda Anguiano, Carmen Aceves.
Instituto de Neurobiología, UNAM. Campus Juriquilla. Boulevard Juriquilla 3001, CP 76230 Santiago de Querétaro, Querétaro. Tel. (442) 238 40 67.
caracev@unam.mx;

The third more frequent pediatric cancer in the world and Mexico is neuroblastoma (NB) which is responsible for 15% of the deaths in children. The first line differentiator agent for the minimal residual disease is all-trans retinoic acid (ATRA), but its use has been limited because is high toxicity at the therapeutic dose. Our group has been demonstrating that the molecular iodine (I2) decreases proliferation and induces differentiation in several cancer cells. The present work aimed to analyze the effect of I2 in combination with therapeutic doses of ATRA in vitro (cell lines) and in vivo (xenografts) conditions. In vitro results show that in SH-SY5Y cell line, ATRA (1 uM) decrease proliferation (MTT assay) and induces differentiation (increase in the neuritic process), and in combination with I2(200 uM) is possible reduce the dose 100-fold maintaining the same effectiveness. In SKN-AS cells, iodide supplement allows to make this cell line sensitive to ATRA at therapeutic doses (1 uM). The synergic responses observed in SH-SY5Y could be associated to increase in differentiation markers expressions like TH, NEFL, TrkA, and MAP2, whereas the I2- sensitivity observed in SK-N-AS cells seems to be secondary to increase in retinoid receptor X (RXR) and peroxisome proliferator-activated receptor gamma (PPARγ) expression (Real-time PCR). In vivo results show that in SH-SY-5Y xenografts (2 cm3 after 3 weeks) from Fox1 nu/nu mice, the drinking water I2 supplement (0.025%) led to reducing the ATRA treatment (i.p. each third day) ten times (1.5 mg/kg BW) maintaining the efficiency; decreases in tumor size (50%) and vascularity (CD31, immunohistochemical) and preventing the side effects; body weight loss and digestive injury (diarrhea). This finding indicates that I2 sensitizes NB cells to ATRA and leads us to propose its evaluation in therapeutic protocols as a possible way to increase effectiveness and avoid side effects.

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Effect of biotin supplementation in the diet on testes cellular proliferation

Tonatiuh Salazar-Anzures[1], Gloria Sicilia-Argumedo[2], Karina Pastén[2], Cristina Fernández-Mejía[1,2]. [1]Unidad de Genética de la Nutrición, Instituto de Investigaciones Biomédicas, Universidad nacional autónoma de México; [2]Instituto Nacional de Pediatría. Avenida del Imán 1, cuarto piso, Colonia Insurgentes Cuicuilco, 04530, Ciudad de México. Tel. 56226420.
crisfern@biomedicas.unam.mx

Introduction: Biotin is a water-soluble vitamin that acts as carboxylase prosthetic group, in addition to this role, at pharmacological concentrations about 30-650 times its recommended diary requirements, biotin modify several biological functions. In previous studies we found that 8 weeks of a biotin–supplemented diet modified pancreatic islet and testes morphology and proliferation. In the testes the expression of the proliferation marker Ki67 was increased; however, it is unknown the mechanisms by which biotin supplementation affects cellular proliferation. In the present work we study the molecular mechanisms that participate in biotininduced testicular proliferation.
Methods: Two groups of three weeks-old male mice were fed during 8 weeks a control diet containing 1.76 mg biotin/ food kg or a biotin supplemented diet 97.7 mg biotin/ food kg (Harlan Teklad, Madison WI, USA). After 8 weeks of feeding, mice were deprived of food for 12 h, anesthetized with Sevorane, and the testes were obtained for protein or RNA extraction. Relative abundance mRNA was analyzed by Q-RT-PCR. Protein expression was assessed by Western blot.
Results: The results showed that, compared to the control group, in the spermatogonia of the biotin-supplemented mice the protein expression of the nuclear proliferation marker PCNA was increased. Western blots analysis of testes protein extracts showed increased abundance of ERK2, as well as augmented ERKp^{Tyr204}, and AKTp^{Ser473} expression without changes of the inactive forms of these proteins. No changes were observed in: the protein abundance of AKTp^{Thr308}; the proliferation receptor CKIT and its ligand SCF. The transcripts of the cell cycle proteins cyclin A2, cyclin E, and CDK2, did not show significant differences between the control and the supplemented mice.
Conclusions: Biotin supplementation promotes spermatogonia proliferation via the canonical proliferation pathways MAPK and PI3K/AKT. The lack of effect on the paracrine transduction signaling CKIT-SCF suggests that biotin-induced proliferation might be mediated by an intracellular signaling.

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Analysis of microRNA expression in formalin-fixed paraffin embedded lung tissue from patients with interstitial lung disease secondary to autoimmune diseases.

Alfonso Salgado Aguayo, Daniel Paz Gómez, Sandra Romero Cordoba*, Miguel Gaxiola Gaxiola, Alfredo Hidalgo Miranda*, Moisés Selman, Carmen Navarro.

*Instituto Nacional de Medicina Genómica
Instituto Nacional de Enfermedades Respiratorias –Ismael Cosío Villegas"
Laboratorio de Investigación en Enfermedades Reumáticas. Calzada de Tlalpan 4502, Col. Sección XVI, C.P. 14080, Tlalpan, México D.F.
Tel. 5487-1700 ext. 5187, email: salgadoa@ymail.com

Background: MicroRNAs (miRNAs) are short chains of RNA that regulate the expression of messenger RNA (mRNA) that have a partially complementary sequence. This interaction may result in the degradation of the mRNA, or in the inhibition of its translation. MiRNAs play an important role in biological processes such as cell differentiation, proliferation and apoptosis; in the immune response, and in various diseases, and as such they have been proposed as therapeutic targets. There are few studies on the role of miRNAs in lung pathology. It has been reported that in idiopathic pulmonary fibrosis (IPF) let-7d is diminished and plays a role in the regulation of epithelial-mesenchymal transition, while miR-21 is increased in patients with this disease; In a model of PF induced by bleomycin, miR-155 correlates with the degree of fibrosis. Patients with rheumatoid arthritis have a modified expression of various miRNAs in the synovium, however there are no studies on the role of miRNAs in lung tissue of interstitial lung disease secondary to autoimmune rheumatic diseases.

Objective: Analyze the expression of miRNAs in lung tissue of patients with interstitial lung disease and validate expression patterns that might help differentiate the underlying disease.

Material and methods. We studied lung biopsies preserved in paraffin from patients with idiopathic pulmonary fibrosis (IPF, n = 8), hypersensitivity pneumonitis (NXH, n = 8), rheumatoid arthritis (RA, n = 7), Sjögren's syndrome (SS, n = 11) and scleroderma (Sc, n = 7). Using a commercial kit, total RNA was recovered from sections obtained from paraffin blocks belonging to patients with the aforementioned diseases. Purified RNA was retrotranscribed and pre-amplified, and the pre-amplified cDNA was used as the template for real-time PCR reactions using the TaqMan Low-Density Array system (TLDA V 3.0, Life Technologies, capable of detecting 754 miRNAs). Ct data were normalized with the endogenous controls RNU44 and RNU48, and compared among groups to reveal expression patterns (i.e. Autoimmune vs not-autoimmune, AR vs SS, AR vs Sc and SS vs Sc). The expression of selected miRNAs was then validated using another RT-PCR approach, the TaqMan Advanced system.

Results: A large number of miRNAs with significantly different expression (adjusted P value <0.05) were detected amongst groups. For example, in the AI vs not-AI comparison up to 20 miRNAs were differentially expressed. The miRNAs were selected to perform an ontology analysis with the genes they regulate and its expression verified with the Taqman Advanced system.

Conclusion and discussion: Each of the studied pathologies presents a particular expression pattern of miRNAs, which could help in the differential diagnosis and open the possibility of developing new therapeutic strategies.

Sex and time differences between kidney histological modifications after acute ischemic/reperfusion injury

Sanchez-Briones M. E., Gonzalez-Alvarado M., and Teran-Hernandez N.
Multidisciplinary Academic Unit Huasteca Zone, Autonomous University of San Luis Potosi
Calle Romulado del Campo #501, fracc. Rafael Curiel CP 79060, Ciudad Valles, San Luis Potosi, telefono: (481)381-23-48 ext. 220 email: eugenia.sanchez@uaslp.mx

Histological changes as leukocytosis, glomerulosclerosis, collapsed glomeruli, tubular brush and tubular dilatation were analyzed in a blind simple trial study in rat kidney (w= 150-200 g, both male n=9 and female n=3). Under general anesthesia (pentobarbital i.p.) the left kidney was dissected and a controlled ischemic period (45 min) was realized. After the reperfusion time assigned by randomization (time for males: 0, 24 and 48 h., for females: 24 h), both kidneys were extracted and 3 micras thickness cuts were obtained, Hematoxilin-Eosin staining and analyzed by a certified pathologist. For ischemic kidneys, our results showed that severe tubular dilatation was found in t24 and severe collapsed glomeruli in t48. Interestingly, in the kidney without ischemia (right) all parameters at all times were mild and moderate. Also, it was found that tubular dilatation and glomerular collapse were more intense in females, while lymphocytosis and brush edge were more intense in males. Tubular dilatation was reported with greater intensity in females compared to males in the control kidneys, a trend that was maintained with respect to ischemic kidneys.
Contrary to expectations, glomerulosclerosis was found to be more intense in the control kidneys in males compared to the ischemic kidney of both sexes and with the controls of the females. IHQ test needs to be probed to establish the relevance of this findings .

Study of the expression of 9-*O*-acetylated sialic acid by the *Macrobraquium rosenbergii* lectin in a cell line of Squamous carcinoma of the oral cavity

Hugo Sánchez Martínez[1], Daniela Illescas Barbosa[2], Victoria Jiménez Castillo[1,3], Cynthia Díaz Hernández[2], Cintya Mayela Félix Rosales[4], Mohamed Alí Pereyra Morales[4], María Concepción Agundis Mata[4], Anayetzin Torres Rivera[2], María Elena Hernández Aguilar[1], Beatriz Xóchitl Ávila Curiel[1], Carlos Josué Solórzano Mata[1,2]*. [1]Faculty of Dentistry and [2]Faculty of Medicine, Autonomous University Benito Juárez of Oaxaca; [3]Technological Institute of Oaxaca; [4]Faculty of Medicine, National Autonomous University of Mexico, Mexico.
Email: universidad99@hotmail.com

Oral squamous cell carcinoma (OSCC) is a common health problem worldwide with an increased incidence among young people. In OSCC, like other malignancies, the glycosylation is a common event that triggers carcinogenesis and tumor progression. The sialic modifications can be detected by lectins and, in OSCC, these changes have been related to the stage, invasion and metastasis. *Macrobraquium rosenbergii* lectin (MrL) specifically recognizes 9-*O*-acetylated sialic acid (9-*O*-Ac-Sia), but how it is modified in tumors and OSCC cell lines is currently unknown. The aim of the present study is to identify the expression of 9-*O*-Ac-Sia using the MrL lectin and the effect over SCC-125 cell line and in HaCaT immortalized keratinocytes. For this purpose, cytochemistry, sialidase specificity, electrophoresis, western blot, proliferation and colony formation assays were performed. Our results showed that MrL recognizes the SCC-152 over HaCaT cells in a precise manner; the specificity of the lectin was demonstrated by inhibition assay with sialidase. The protein electrophoresis profile was similar for both cell types, however, the western blot showed differences in the glycoprotein pattern. In addition, MrL increased the proliferation in SCC-152 cells but not in the colonies count, in fact, these colonies maintained their morphology and integrity, whereas the HaCaT cells did not exhibit changes. Our results showed that 9-*O*-Ac-Sia is expressed in SCC-152 cells, and their implication in the proliferation and survival of OSCC cells.

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Key words: 9-*O*-acetylated sialic acid, squamous carcinoma of the oral cavity, *Macrobraquium rosenbergii* lectin.
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Alteration in cell viability, DNA damage and changes in Hsp70 expression in human leukocytes exposed to UVA light and heat.

David Alejandro García López, Jennifer Ortiz Letechipía, Maricela Olarte Saucedo, Rosa Gabriela Reveles Hernández, Rosa María Ramírez Santoyo, Luz Elena Vidales Rodríguez, María Argelia López Luna y Sergio Hugo Sánchez Rodríguez.
Universidad Autónoma de Zacatecas.
98600, 4921052997, smdck@hotmail.com

SUMMARY

Introduction. Skin cells in humans suffer greater stress due to continuous exposure to solar radiation, of which the most stressed are ultraviolet (UV) and heat. UV radiation is classified by its wavelength into: UVA, UVB, and UVC. UVA light is absorbed by 70 to 80% of the cells of the dermis and melanocytes of the basal epidermis and is the main factor generating oxidative stress in skin cells, affecting proteins, deoxyribonucleic acid (DNA) and lipids. To maintain homeostasis due to UV stress, changes have been observed in the expression of heat stress proteins, which help protein folding under normal conditions and during stress renature them or induce their destruction when the damage is greater.

The objective of the work was to determine cell viability, DNA damage as well as the expression of HSP70 in human leukocytes exposed to UVA light and heat.

Material and methods: Leukocytes from healthy human patients were separated from whole blood by the Ficoll Histopaque® method. Once this was done, viability was measured at the time of production and after UV and heat stress. Subsequently, the leukocytes were exposed to heat stress (37, 40, 42 and 44 ° C) for one hour, and to UVA light (λ 365nm) one, two and three hours. Once the above was done, DNA was extracted by the DNAzol® method, DNA fragmentation was evaluated on agarose gels (0.8%). Likewise, the proteins were quantified by the Bradford method, the Hsp70 protein was analyzed with a specific antibody by PAGE-SDS and Western Blot-ECL.

Results. A non-significant decrease in the viability of leukocytes exposed to UVA light was found at 1, 2 and 3 hours of exposure, and with heat at 40, 42 and 44 °C. What is relevant is that a significant decrease in the total number of cells was found by both stressors. An increase in the expression of Hsp70 was also observed by heat at 40 and 42 °C and by UVA light at one hour and two hours of exposure. Likewise, DNA fragmentation was found in the samples exposed to two and three hours in UVA light and a discrete fragmentation at one hour with UVA light and at 44 °C.

Conclusion. UV light and heat generate cellular stress by altering cell viability, Hsp70 expression and DNA damage in human leukocytes.

Isolation and purification of Extracellular Vesicles from breast cancer cell lines MDA-MB23 and T47D

Alvaro Adrian Sandoval Montiel, Paulina Cortés Hernández, Ileana Conde Rodríguez, Jonathan Guerrero Reyes, David Medina Benítez, Cynthia Paola López Pacheco, Maricruz Anaya Ruiz, Nora Hilda Rosas Murrieta*.
*Centro de Química ICUAP, Benemérita Universidad Autónoma de Puebla, Edificio 1IC-7, Ciudad Universitaria, Puebla CP72570. Tel.: (222)2295500 ext. 7283 correo: nora.rosas@correo.buap.mx
CIBIOR, HGZ NO.5 IMSS Metepec-Atlixco Puebla, Carretera Atlixco-Metepec Centro, CP 74360.

In the past decade, extracellular vesicles (EVs) have been recognized as potent vehicles of intercellular communication, both in prokaryotes and eukaryotes 1. This is due to their capacity to transfer proteins, lipids and nucleic acids, thereby influencing various physiological and pathological functions of both recipient and parent cells, but this specificity needs to be clarified yet. EVs on normal cells are classified as: exosomes sizes range from 30-120 nm, and microvesicles range from 100-1000 nm. They have a bilipidic layer, with integrated membrane proteins as tetraspanin (e.g. CD63, CD81, CD82, CD53, CD37 and CD9), integrines. Tumoral cancer cells as normal cells can produce the same type of Evs, as described above. As in normal cells EVs from cancer cells have the same role in transfer horizontal information and modify the microenvironment, however the role of EVs from cancer cells go far away from this. They are able of transforming the microenvironment to prepare metastasis, reprogramming cells to help them in their invasiveness, and their cell's fate. EVs from cancer cells can propagate oncogenic information, including transfer of signal transduction complexes, across tissue spaces. EVs, such a horizontal transfer mechanism, can deliver transforming signals of many kinds throughout tissues and even to distant sites, that could the main key in the metastasis development and drug resistance. EVs could explained at last, some poorly understood phenomena, for instance intravasation and extravasation, tumor dormancy, distant cell reprograming, preparation of distant sites before implantation of metastatic cells, transferring drug resistance, inducing angiogenesis. EVs have been studied since decades ago, however the roles of these vesicles in normal cells were underestimated, even in cancer cells. In the past decade, it has been a when we begin to understand the main role that EVs play on the cellular homeostasis, breaking the paradigm that information across the cells was mainly due to cell to cell connections. Now, we know, that this information goes in a horizontal pathway from parental to recipe cells, regulating on this way growth, differentiation, etc. Even, important the role of cancer cells-derived EVs, has been taking a key play in understand the development of this disease. Discovering the role of EVs in cancer development will be a tremendous tool, allowing the develop of novel therapies. Cancer cell-derived EVs are a promising target for therapeutic intervention. Isolation actually is a main key on the study of EVs and there's diverse techniques to obtain them. In the present work we isolated ant purified EVs from Breast cancer cell lines MDA-MB231 (triple negative) and T47D (Strogen receptor positive). Each cell line was growth in 75 cm^2 cell culture flask, until 70% confluence, at this point cells were washed with PBS and appropriated medium without FSB was added (30 mL) y

culture during 72 hrs. After this time medium was recovered and centrifugated 1x for 800 rpm for 5 minutes to discard free cells, supernatant was recovered centrifugated 1x for 1500 rpm for 5 minutes to discard apoptotic bodies and detritus. Supernatant was recovered. These media prepared for ultracentrifugation 100,000 xg for 1hr at 4ºC. After centrifugation bottom was recovered (note that no pellet was observed) in 2 mL of medium. To avoid large EVs the medium with EVs was filtered with a 0.22 □m filter unit. 1 mL was aliquoted and used with Nanosight NS300. Results: **EVs231** 1.22 x 10^9 particles/mL, media size 134.4 nm +/- 3.2 nm. **EVsT47D** 3.98 x 10^8 particles/mL, media size 352.9 nm +/- 73.3 nm. Conclusions: EVs from cancer cell lines is a simple protocol, however isolation and purification are necessary the use of ultracentrifugation that requires that a laboratory with the infrastructure and it's also a consuming time process. Vesicle performance its apparently adequate and these vesicles can be used for more in-depth studies.

Characterization of a cell line with mesenchymal appearance possibly differentiating into cancer associated fibroblast from a FeNTA-induced RCC tumor.

José Dolores Solano Becerra1*, Telma Olivia Pariente Pérez1, Patricia Curiel Muñiz1, Francisco Antonio Aguilar Alonso1, María Elena Ibarra-Rubio1*.
1Departamento de Biología de la Facultad de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, CDMX, México. CP 04510, * jsolanob2000@yahoo.com , meir@unam.mx

Renal cell carcinoma (RCC) is the most common renal neoplasm in adults, represents 2 to 3% of all tumors, its progression is asymptomatic even in very advanced stages, its diagnosis is tardy and its mortality rate is high, so the study during its progression is very limited. For all the above an *in vivo* experimental model of RCC induction in rats initiated with N-diethylnitrosamine (DEN) and promoted with ferric nitrilotriacetate (FeNTA)1 is a useful tool to analyze renal cancer from its early to the most advanced stages.

In recent years the role of the tumor microenvironment (TME) in the carcinogenesis process is being investigated, the TME contributes to the progression and migration of tumor cells and additionally affects the efficacy of the treatment. Therefore, has been proposed to consider to tumor stroma a therapeutic target, however, it is still necessary to characterize the role of the cellular participants that make up the TME and which contribute among other actions with a remarkable process of transdifferentiation known as epithelium-to-mesenchymal transition (EMT).

Additionally, studies have found a correlation between the presence of cells with an EMT phenotype in tumor tissue from RCC with survival, and renal fibrosis in patients with RCC with survival, in both correlations a very poor prognosis is detected2. For all these reasons, obtaining a cell line with an EMT phenotype from RCC tumors induced by FeNTA could be exposed to treatments with drugs currently used in the clinic, or with candidates for antitumor agents. Said obtaining was already achieved in our working group, through a series of passes (75) from a primary culture and a subsequent cloning by limiting dilution, the results of a preliminary phenotyping indicate that this line is probably differentiating towards fibroblasts, in this case to fibroblasts associated with cancer.

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Determination of polycyclic aromatic hydrocarbons in a university population

Samantha Daniela Suárez Pérez, Carlos Mario Morales Bautista, Blanca Estela Trejo Sánchez, Guillermo Segura Vázquez, José Arnold González Garrido, Carlos Javier López Victorio. Universidad Juárez Autónoma de Tabasco, Carretera Cunduacán-Jalpa KM. 1 Col. —la Esmeralda" PC. 86690, 5539965215, Javier.lopez@ujat.mx

Objective:

To correlate the presence of molecules with aromatic rings in urine of obese and nonobese patients.

Introduction: Polycyclic aromatic hydrocarbons (PAHs) are organic compounds. The PAHs and their derivatives are found in the environment as contaminants. HAP and are produced mainly from the partial combustion of fossil fuels (biogenic, petrogenic and pyrogenic). Among the pyrogenic PAHs is the anthracene used in the production of anthroquinone, an important raw material for the manufacture of rapid dyes, and the phenanthrene that is used in the manufacture of dyes, explosives, in clinical research and the synthesis of drugs. Recently, it has associated PAH with the development of metabolic diseases such as obesity, hypertension and also kidney diseases(1)**.** Obesity (Ob) belongs to chronic noncommunicable diseases (CNCD) that together with diabetes, hypertension, dyslipidemias, chronic kidney disease, represent more than 80% of deaths, the major causes of disabilities and health costs with great impact on society (2) so that its determination in biological samples is important as a control strategy of the Ob.

Methodology: Anthropometric measurements; weight, height, determination of body mass index (BMI) (3), abdominal circumference (CA), hip circumference (CC), urinalysis, identification of aromatic rings by infrared spectrometry at 270 - 291 nm, specialized surveys after authorization of informed consent.

Results: In this preliminary study 155 individuals participated, all students between 19 -22 years of age, we determine that 52.25% of the participants present Normal weight, 27.09% overweight and 20.64% any type of Obesity, being 87.5% of the sex male and a 12.5 female.

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Analysis of cellular genes expressed in extracellular vesicles (EV) from the HeLa cell line

Tello-Ortega Karla Esmeralda[1], Mata-Rocha Minerva[2], Romero-Tlalolini María de los Angeles[1]; [1]Universidad Autónoma Benito Juárez de Oaxaca (UABJO); [2] Hospital de Pediatría, CMNSXXCI; Correspondence: UABJO, C.P. 68020, 55 13 82 47 68, mdlaromerotl@conacyt.mx

Introduction

Cervical cancer is a serious health problem worldwide. In Mexico is the second cause of death due to malignant neoplasms. Some studies have shown that the production of extracellular vesicles (EV) is higher in cancer cells than in healthy cells. These EV can be a conveyance with information that allows the communication between cancer cells and the healthy tissue proximal. Therefore, they could promote the cancer by transporting oncogenic factors or other molecules which modify the disease or the therapeutic resistance (Bebelman, et al., 2018; Steenbeek et al., 2018). Accordingly, our main goal is to analyse the presence of cellular transcripts expressed in EV from the HeLa cell line comparing with keratinocyte cell lines without HPV infection.

Methodology

We analysed the transcripts detected in EV from three cell lines HeLa, HaCaT and C33-A from a massive sequencing of RNA. Six transcripts expressed mainly in EV of HeLa cell line (including messengers, micro, and long non coding RNAs) were selected and evaluated by RT-PCR in order to confirm the results.

Results

We found 103 transcripts mainly expressed in RNA of EV from the HeLa cell line and 5785 genes present mainly in healthy keratinocytes. We select the transcripts of four cellular genes (S100A9, HBB, FTL and TMSB4X) one miRNA (mir6087) and one lnc-RNA (RMRP), and confirm their presence in such EV. This molecules could serve as possible molecular markers for cervical cancer in extracellular vesicles.

Conclusions and Impact

The EV from HeLa cell lines contains several transcripts which are mainly expressed in this HPV positive cell line, these transcripts correspond to messenger RNAs, microRNAs, and lncRNAs. They could have an effect on the cancer or the therapeutic response, but this need to be proved.

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Histone deacetylase-6 inhibition reduces the profibrotic effects of bleomycin and TGF-β in mice lung.

María Fernanda Toscano Márquez[2], Soledad Aguirre Garduño[2], María de los Ángeles García Vicente[2], Iliana Herrera Fuentes[1], José G. Cisneros Lira[1,3].
[1]Laboratorio de Biología Celular, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas. México D.F. [2]Posgrado en Ciencias Biológicas, UNAM. [3]Laboratorio de Fibrosis-1, LaNSBioDyT UNAM.
Departamento de Investigación en Fibrosis Pulmonar, INER; Calzada de Tlalpan 4502, Col. Sección XVI CP 14080, México D.F. 54871700 Ext. 5257 jcisneros828@gmail.com

Lung fibrosis is the final stage of several pulmonary diseases and is characterized by abnormal collagen deposition which leads to the destruction of pulmonary architecture. Histone deacetylases (HDACs) are highly conserved enzymes involved in chromatin remodeling and have important roles in different diseases, and several studies suggest that the non-selective inhibition of HDACs ameliorates the progression of pathological processes involved in cancer or fibrotic diseases. However, there are few reports the show the effects of selective HDACs inhibitors, and in this sense, the role of HDAC6 in the development and progression of lung fibrosis remains unclear. We aim to investigate the role of the Tubastatin A, a selective HDAC6 inhibitor, on cellular processes associated with the development of pulmonary fibrosis.

Lung fibrosis was induced by instillation intratracheal of bleomycin in mice C57BL/6, and the animals were treated daily with the selective inhibitor Tubastatin A (TubA) and were euthanized at 7, 21 and 28 days after bleomycin instillation. Lung tissues were used for histological analysis of inflammation, fibrosis, expression of profibrotic and inflammatory molecules, and collagen content. Bronchioalveolar lavage was used to analyze inflammatory cytokines, and lung primary fibroblasts isolated from mice treated with bleomycin and/or TubA were used to analyze proliferation and migration. Additionally, the involvement of HDAC inhibitor on apoptosis, proliferation, migration, and the expression of matrix remodeling enzymes MMP-2/MMP-9, were assayed in mouse lung fibroblasts (CCL-206, ATCC) treated with the profibrotic molecule TGF-β.

Results showed that TubA treatment attenuates the inflammatory response induced by bleomycin by decreasing neutrophil infiltrate, IL-6 and NF-kB expression. Histology results showed a correlation between a higher number of H3K9K16ac positive cells and a lower grade of lung damage and extension of fibrotic areas as well as a significant reduction of TGF-β, ACTA2, and lung collagen in those animals treated with TubA compared with bleomycin alone. The proliferative and migrative feature of the fibroblasts isolated from lungs of mice treated with bleomycin was significantly decreased with the treatment of TubA. Similar results were observed in CCL-206 fibroblasts and on mouse epithelial cells. Finally, we observed that TubA reduced the pro-fibrosing effects of TGF-β since it decreases the proliferation and activity of MMP-2 and MMP-9.

These results suggest a protective role of TubA in the pathogenesis of pulmonary fibrosis, and our findings in fibroblasts demonstrate a critical role of histone modifications in the fibro proliferative-migratory signature in this disease.

Prevalence of overweight and obesity in university students of the Chontalpa

Blanca Estela Trejo Sánchez, José Ángel Jerónimo Alejo, José Arnold González Garrido. Laboratorio Clínico de Ciencias Básicas, División Académica de Ciencias Básicas, Universidad Juárez Autónoma de Tabasco. Carretera Cunduacán-Jalpa KM. 1 Col. La Esmeralda CP. 86690. Tel. (993) 3581500 Ext. 6702. Cunduacán, Tabasco, México. blanca.trejo@ujat.mx

The abnormal or excessive accumulation of fat triggers overweight or obesity, a situation that is currently affecting a large part of the world population. Until October 2016, the WHO (World Health Organization) has a record of more than 1.9 billion adults over 18 years of age or over, of whom more than 650 million were obese. These figures indicate that 39% of this population (39% of men and 40% of women) were overweight (body mass index (BMI)> 25 <30). Regarding obesity (BMI> 30), we have data that 13% of the world population (11% of men and 15% of women) were obese. It is also reported that between 1975 and 2016 there has been an increase, more than three times, in the prevalence of obesity. In the south-southeast region, the reports described are insufficient to describe the state of our population. Therefore, in this work we determined the prevalence of overweight and obesity as well as their correlations with biochemical parameters in university students. A prospective study was carried out in the Clinical Laboratory of Basic Sciences in the period from February 2015 to December 2017. After signing informed consent, blood samples and anthropometric parameters were obtained, as well as the determination of biochemical parameters (Glucose, Cholesterol and fasting triglycerides of 12 hr) in new students to the Universidad Juarez Autonoma de Tabasco (UJAT). We evaluated the prevalence of overweight and obesity based on the body mass index (BMI) in the IBM SPSS program. Of a population of 4755 individuals, with an average age of 18 years, of which 14.5% of women presented obesity and 24.9% were overweight. On the other hand, the prevalence of obesity in men was 17.1%, while in overweight it was 29.4%. For both genders it is observed that during the years 2015 and 2016 the pattern of prevalences in overweight and obesity is maintained; however, in 2017 a significant increase was observed. From the biochemical parameters it is observed that the measurements of triglycerides in the obese group exceed the normal limit. These results emphasize that obesity and overweight are still a major problem in the Mexican Southeast. Its recommended that in order to control these situations, emphasizing and following up prevention programs.

Role of the Endogenous Opioids in the modulation of the Expression of Opioid Grow Factor Receptor (OGFr) and Transient Receptor Potential Vanilloid 1 (TRPV1) In a Rat Alkali-Burned Cornea Model.

*Eduardo Emmanuel Valdez-Morales; Andrea Estefanía Sánchez-Valdez; Luis Fernando Torres-Bernal; Rafael Villafan-Bernal; Tonatiuh Barrios-García; Sugela Susana Blancas-Zugarazo; Raquel Guerrero-Alba.
Universidad Autónoma de Aguascalientes, Departamento de Cirugía, Centro de Ciencias de la salud, Av. Universidad # 940, Ciudad Universitaria, C. P. 20131, Aguascalientes, Ags. México.
*qbvaldez@gmail.com

Introduction: The cornea is the most anterior structure in the eye, it has the property of being transparent and allows access of light inside the eye. Severe cornea injuries like alkali burns can result in loss of its transparency, causing deficient vision or total blindness. It has been proposed that cornea wound healing might be regulated positively by some ion channels like TRPV1. Moreover, it has been described that TRPV1 activity can be down regulated by classic opioids receptors, mu (μ) delta (δ) kappa (κ). However, there is no evidence if OGFr, a non-classical opioid receptor which has been proposed like a negative tissue grow agent, can modulate TRPV1 activity as well.

Aims: In this study we sought to investigate cellular expression of both, OGFr and TRPV1 in corneal tissue and trigeminal neurons after alkali-burned injury.

Methods: We used Wistar rats of 200-300 grams of weight under general anesthesia with *zoletil 50* 25mg/Kg. Alkali burns were generated in both eyes with 6 mm of diameter filter paper soaked with NaOH 1N, then placed 45 seconds on each cornea, ophthalmic strips with sodic fluorescein were used as indicator of damage under cobalt blue light. Four groups of rats where made: First group was control, second group was control plus 10μM Naltrexone, third group was alkali burned and the fourth group was alkali burned plus Naltrexone. For groups 3 and 4, corneas were collected at 0, 24, 48 and 72 hours after alkali-burned injury. Control cornea group with or without naltrexone were kept intact and collected in the same time course. The corneas were placed in paraformaldehyde for histology or TRIZOL®reagent for gene amplification. Tissue integrity was evaluated by hematoxylin/eosin (HE) preparations. Trigeminal cell culture was prepared from controls rats and then, the cells were incubated with 100nM Met^5Enkefalin and/or 10μM Naltrexone. OGFr and TRPV1 expression was examined by using endpoint RT-PCR

Results: In alkaline burned corneas, HE preparations shown minimal changes at 0 hours in tissue integrity, while at 24 hours was observed an epithelium thickness diminished with stroma crosslinking and a marked inflammatory cell infiltration, this behavior was similar up to 48 hours, however at 72 a slight reepithelization was observed. Moreover, we were able to amplify OGFr and TRPV1 in corneal tissue as well as in trigeminal neurons by RT-PCR, detecting in a preliminary way changes in TRPV1 expression in alkali burned corneas as well as in trigeminal neuron under incubation with 10μM Naltrexone.

Conclusion: Reepithelization of burned corneas takes place at 72 hours after the injury. Cellular infiltration in corneal tissue decreases. The OGFr and TRPV1 channel is expressed in the rat burned corneas and controls, however, it seems that these receptors are being regulated by endogenous opioids.

PARTIAL CHARACTERIZATION OF PEPTIDE/ PROTEINS WITH ANTIMICROBIAL ACTIVITY IN THE *Scolopendra viridis* VENOM

Lucero Valladares Cisneros[1], Carolina Abarca Camacho[1] and María del Carmen Gutiérrez Villafuerte[1]. [1]Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos Av. Universidad 1001, Col. Chamilpa. Tel: (01)7773297057 ext. 3522.
carmengu@uaem.mx

Antimicrobial proteins and peptides (APPs) identified up to this day have been classified basically according to its charge, as cationic or anionic. Most of the attention they have received relates to the fact they can be excellent candidates for the development of new antimicrobial agents. A number of the resulting peptides are now examined in clinical trials at various stages, but were not yet approved for therapeutic applications. Many APPs from arthropods such as insects and scorpions have been thoroughly studied (1); in the venom of the centipede *S. subspinipes mutilans*, there are reports of several peptides with both antibacterial and antifungal activities. (2, 3)., Our group has recently reported a 13 kDa antimicrobial peptide from *S. polymorpha* venom (4). Thus, the aim of this study is to identifier if *S. viridis* venom also contained APPs.

S.viridis venom extraction was performed by mechanical stimulation of the forciples and protein was quantified by the Lowry method. Venom was separated by RP-HPLC, collecting fractions every 5 min; by anion exchange chromatography obtaining 5 fractions and by 6% SDS-PAGE of whole and fractions venom.

Antimicrobial activity of venom and fractions venom were evaluated by either agar diffusion method or direct agar diffusion by SDS-PAGE, according to León *et al*. (5); briefly, after electrophoresis, the gel was aseptically cut in half, one of which was stained with Coomassie blue to identify the protein pattern, and the other half was rinsed with ethanol and TRIS- HCl and then placed directly over the agar plate, where *S. aureus* (ATCC 29213) was previously inoculated (at various concentrations, from

0.070 to 0.13 O.D., measured at λ 600 nm). Once the active peptide/protein(s) were identified, a second 16% SDS-PAGE was carried out, to determine their molecular weight and to cleave those specific bands of interest, prior to their electroelution. Antimicrobial activity was then re-assessed by agar diffusion method, using ampicillin as positive control (5µg/µl), at 37°C. Finally, we also tested *in vitro* hemolytic activity on human erythrocytes of the active peptides/proteins, using Triton X-100 as positive and PBS as negative controls. We found 4 anionic proteins with antibacterial activity vs. *S. aureus* (ATCC 29213), with molecular weights of 26, 40, 60, and 118 kDa. The 40 kDa peptide showed the greater antimicrobial activity, at a concentration of 1.5 µg/3µl, and produced 20.5% of hemolysis. Its partial amino acid sequence was determined.

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Inverse correlation between levels of glycosylated hemoglobin and SERCA protein expression levels in patients with type 2 diabetes mellitus

Jose Gustavo Vazquez-Jimenez[1], Rodrigo Martinez-Corella[1], Jesus Rene Machado-Contreras[1], Moises Sandoval Lopez[2], Eleobardo Castro Luque[2] and Octavio Galindo-Hernandez[1].* [1]Facultad de Medicina, 1Universidad Autonoma de Baja California, C.P. 21000, Mexicali, Baja California, Mexico. [2]Hospital General 5 de Diciembre ISSSTE, Mexicali, Baja California. *gustavo.vazquez@uabc.edu.mx, Tel. 55-42-22-76-25.

Insulin resistance is a systemic condition in which cells ability to respond to this hormone decrease1. So, if the organism does not respond effectively to insulin actions, blood glucose levels increase, inducing the onset of type 2 diabetes mellitus (T2DM), which represents a serious public health issue[1].

Since the endoplasmic reticulum (ER) is a specialized organelle responsible for synthesis, folding and assembly of secretory and membrane proteins, there are solid data demonstrating that endoplasmic reticulum stress (ERS) is one of the main causes of insulin resistance genesis[2]. Given that the ER needs a high luminal calcium concentration to function properly[2], the sarco/endoplasmic reticulum calcium ATPase (SERCA) plays a key role, since it is responsible of maintaining high levels of calcium in the ER by its recapture. Several reports have shown that decreased expression and activity of SERCA pump induces ERS[3]. Perturbation of ER homeostasis leads to stress and activation of unfolded protein response (UPR), maladaptive ER stress leads to the activation of JNK, which has been suggested to be involved in decreasing the insulin signaling pathway, which in turn results in the corresponding insulin resistance condition[3].

In congruence, overexpression of SERCA protein has been shown to decrease the insulin resistance state in cell cultures and in rodents [3,4]. All those features mentioned above could place SERCA as a possible therapeutic target.

In the present work, we have studied SERCA expression levels in platelets of apparently healthy people and T2DM patients. The results showed that, as in the state of insulin resistance, the levels of SERCA expression are diminished in T2DM patients.

Moreover, when classifying the samples according to the different levels of glycosylated hemoglobin in ascending order, SERCA expression levels progressively decreased until being undetectable. In summary, the findings demonstrate that decrease in SERCA protein levels is progressive and depends on the level of uncontrolled T2DM.

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Advanced glycation end products levels in preeclampsia and their implications ininsulin resistance

Edgar Ricardo Vázquez-Martínez, Mariana Gabriela Bobadilla-Bravo, Elizabeth García-Gómez, Sonia Nava-Salazar, Eulises Díaz-Díaz, Yessica Torres-Ramos, Ignacio Camacho-Arroyo, Marco Cerbón*
Instituto Nacional de Perinatología-Facultad de Química, UNAM
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán
*Corresponding author. Unidad de Investigación en Reproducción Humana, Instituto Nacional de Perinatología-Facultad de Química, UNAM. Ciudad Universitaria, Av. Universidad 3000, Coyoacán 04510, Mexico City, Mexico. Tel. 55209900 ext. 513, email: mcerbon85@yahoo.com.mx

Background. Advanced glycation end products (AGEs) are glycated biomolecules from both exogenous and endogenous sources. The accumulation of these compounds in the body contributes to cellular damage at the vascular system, and especially alters the metabolic responses mediated by insulin. There are few studies about AGEs levels during inflammatory obstetric disorders unrelated to hyperglycemia, such as in preeclampsia (PE). Therefore, the aim of the present study was to determine the circulating levels of maternal AGEs in preeclampsia and to evaluate their possiblecorrelation with clinical parameters of glucose metabolism.

Methods. All samples were obtained from women with 36-40 weeks of gestation after obtaining informed consent. Plasma levels of total AGEs were determined by competitive ELISA (Lamider, Mexico) and plasma levels of intact albumin-AGE complexes were measured by radioimmunoassay (RIA) in women with normoevolutive pregnancy (control group, n=28) and in patients diagnosed with PE (n=15). We also evaluated the correlation between levels of AGEs and metabolic profiles of the preeclamptic patients.

Results. Plasma levels of total AGEs in patients with PE (29.66±2.99 µg/mL) were significantly higher ($p<0.05$) than those observed in the control group (19.68±2.41 µg/mL), while the levels of intact albumin-AGEs complex did not show significant differences between control and PE group. A positive correlation between the circulating levels of total AGEs and insulin ($r=0.61$), glucose ($r=0.62$), and HOMA ($r=0.74$) levels was observed.

Conclusions. In the present study, we have demonstrated that plasmatic total AGEsare increased in PE normoglycemic patients. In contrast, we did not find statistical differences in the content of intact albumin-AGEs complexes between PE patients and control group, which suggests a probable increase in the activation of AGEs degradation system. Interestingly, we also found a positive correlation of insulin resistance with AGEs levels even in normoglycemia, suggesting that glycation-promoting mechanisms may affect signaling pathway of insulin.

Intermittent Fasting plus Moderate Exercise Decreases Metabolic, Inflammatory and Cardiovascular Alterations Experimental Diabetes Induced.

Adolfo Virgen Ortiz1, Sarai Limon Miranda2, Alejandro Apolinar Iribe3, Johana Garcia Vaca1
1Universitary Center for Biomedical Research, University of Colima, Colima, Mexico. C.P. 28045.
E-mail:
avirgen@ucol.mx
2Deparment of Chemistry-Biological Sciences and Agropecuarys, University of Sonora, Navojoa, Sonora, Mexico.
3Physics Department, University of Sonora, Hermosillo, Sonora, Mexico.

Diabetes is a metabolic disease characterized by hyperglycemia that results from a decrease in insulin or insulin resistance. Diabetes is a disease of high prevalence worldwide that affects the quality of life of people due to various complications that compromise their health and lead to their death. The objective of this study was to evaluate the effects of an intermittent fasting intervention program plus moderate exercise on the progress of the pathogenesis of experimental diabetes. Male rats of the Wistar strain were divided into 4 groups: healthy rats, healthy rats plus fasting and exercise, diabetic rats and diabetic rats with fasting and exercise.
Diabetes was induced by the intraperitoneal administration of nicotinamide (120 mg/kg) followed by streptozotocin (65 mg/kg), a week later hyperglycemia was confirmed with a glucometer. An intervention program was applied for 3 months which consisted of an intermittent fasting (12 hours without food and 12 hours with free access to food) and a protocol of moderate exercise consisted in running on treadmill (intensity equivalent to an oxygen consumption of 60- 70%). The parameters evaluated were fasting blood glucose triglycerides, IL-1β, TNF-α, IL-6 and systolic, diastolic and mean pressure. The data analysis was performed with the software Sigmaplot 12. The combination of intermittent fasting and moderate exercise for 3 months attenuated the development of the pathogenesis of diabetes, causing a reduction in blood glucose levels, triglyceride levels, cytokines inflammatory and attenuating the increase in blood pressure (systolic, diastolic and mean) in an experimental model of type II diabetes in rats. The application of an intervention program of intermittent fasting plus moderate exercise induces beneficial effects by preventing the progression of the pathogenesis of type II diabetes.

Activation of local intracardiac reflexes by mechanical stimuli in the rat heart endocardium.

Sharon Zayuri Zenteno de los Santos (post-graduate student); María de Jesús Gómez Villalobos.
Institute of Physiology, BUAP
Prolongación de la 14 Sur 6301, Cd Universitaria, Jardines de San Manuel.72592 Puebla, Pue. Tel: (222)2295500 Ext. 7308 y 7312. mgomezvillalobos@aol.com

Cardiovascular reflexes are systemic homeostatic mechanisms that are explained with the participation of the central and peripheral nervous system, however, there is evidence that local intracardiac reflexes can be generated, independently of these structures, which could participate in the regulation of heart functions. In this study we propose to demonstrate the generation of local intracardiac reflexes in the isolated heart of the rat. Wistar Kyoto (WK) rats of both sexes and 3-4 months old were used. After general anesthesia, the heart was rapidly removed and perfused with Krebs-Henseleit solution at a flow rate of 10 ml/min and temperature of 37 °C, continously gased with carbogen in a Langendorff system. During the experiment, heart rate (HR), right ventricular (RV) and left (LV) systolic pressure, and $dP/dT_{max\ and\ min}$ were monitored. Preload changes were made in RV and pressure record in the LV, and vice versa (0 to 400 µl, every 3 minutes) in intact hearts and with right atrial (RA) section.The results show that the preload changes in the RV generate a depressive response in the heart rate in a significant way (baseline HR in beats/min of 231±16.64 vs 151±27.4, p> 0.05), effect that was increased in relation to direct to the increase of preload, observing a recovery of the HR when returning to the normal preload. The decrease in HR was associated with sinus bradycardia and transient complete atrio-ventricular block. When changes were made in the preload in the LV, a variable response was observed and less effect than observed in the RV (baseline HR 236.01 ± 31.95 vs 221.79 ± 9.69; NS) and in fact with the first preload change, a stimulatory response was observed (baseline HR 236.01±31.95 vs 256.51±19.44; NS) and with greater presence of premature ventricular contraction and not sustained ventricular tachycardia. With regard to ventricular pressure and $dP/dT_{max\ and\ min}$, right and left, a tendency toward decrease was observed with changes in RV and LV preload, respectively, however they were not statistically significant. When the right atria section was performed, the depressant response in the HR was abolished. The results show that stretching of the endocardium activates a local reflex in the isolated heart, which involves the right atria and particularly the endocardium of the right ventricle, in which the intrinsic nerve structures of the heart could be participating.

Molecular detection of four common species of *Candida* from blood cultures

José Oscar Arturo Hernández Carreón, Alejandro de las Peñas Nava, Irene Castaño Navarro*
*Instituto Potosino de Investigación Científica y Tecnológica. Camino a la Presa San José #2055, Lomas 4a Sección C.P. 78216, San Luis Potosí, S. L. P. e-mail: icastano@ipicyt.edu.mx Tel. (444) 834 2063 Ext. 2038

Candidemia is the fourth cause of nosocomial infections in the bloodstream and is associated with high mortality. More than 90% of candidemia cases are caused by only four species, *C. albicans, C. glabrata, C. parapsilosis and C. tropicalis*. The treatment depends on the species due to the intrinsic resistance to certain antifungals by some species, however, the methods used today for the identification of each species are slow and imprecise.

We used oligonucleotides designed at IPICYT, to amplify species-specific sequences of the main species causing candidemia and we determined the distribution of *Candida* species in 194 clinical isolates from the National Institute of Medical Science and Nutrition Salvador Zubirán (INCMNSZ) and the National Institute of Cancerology (INCAN) from August 2015 to May 2017, we found that *C. glabrata* corresponds to 31.4% of the cases, *C. tropicalis* to 23.7%, and *C. parapsilosis* to 8.2%.

We tested two commercial kits and a homemade method for the extraction of DNAg from blood cultures positive for *Candida*. The homemade method described by Ausubel (**FP**) proved to be the most suitable for the extraction of DNAg. With this method we detected 105 cells/mL with a sensitivity and specificity of 100%.

With this method we also detected 105 cells of *C. glabrata* and *C. albicans* and 104 cells of *C. tropicalis* and *C. parapsilosis* directly from whole blood samples.

We also analyzed genetic relatedness using RAPD-PCR analysis with 39 *C. glabrata* isolates from 15 different patients. We found six different genotypes of which genotype **I** was the most prevalent since it was found in clinical isolates from six different patients and some genotypes were only found in one patient. All the isolates from the same patient showed the same genotype.

Finally, we found that 51.3% 19/37) of the *C. glabrata* isolates were Fluconazole resistant in a Dosage-dependent manner (FLC^{dd}), 45.9% were sensitive to FLC and only one isolate was FLC resistant. In addition, two isolates displayed a decrease in sensitivity to FLC with respect to the other isolates from the same patient.

Los niveles circulantes de plasmalógenos se asocian con el hígado graso no alcohólico.

Marisol Olivares Arévalo **Adscripción:** Unidad de Genómica de Poblaciones Aplicada a la Salud, UNAM-INMEGEN **Institución:** Instituto Nacional de Medicina Genómica **Dirección postal:** Oriente 245 No. 151, Col. Agrícola Oriental, Del. Iztacalco, Cd.Mx. C.P. 08500 **Correo electrónico:** solmar.0413@gmail.com

Introducción: El hígado graso no alcohólico (HGNA) se define con la acumulación de lípidos en más del 5% de los hepatocitos en ausencia de consumo significativo de alcohol u otros factores que afecten al hígado, se relaciona con la obesidad, siendo más prevalente en población latina (LaBrecque, *et* al, 2012; Vega-Badillo, 2016). La entidad patógena es la esteatohepatitis no alcohólica (EHNA), en donde además de la acumulación de grasa (esteatosis), se presenta un estado inflamatorio, pudiendo derivar en cirrosis o hepatocarcinoma. Las herramientas de diagnóstico no invasivas no permiten diferenciar entre esteatosis y EHNA, por lo que el estándar de oro es la biopsia hepática (Castro, *et al*, 2015; Buzzetti, otros, 2016; Friedman, *et al*, 2018). Los trabajos en lipidómica sobre HGNA han identificado tipos de glicerolípidos, colesterol, ácidos grasos poliinsaturados y fosfolípidos que podrían ser biomarcadores séricos útiles en el diagnóstico de estadios más avanzados; aunque no han sido confirmado en cohortes étnicas diferentes (Puri, *et* al, 2009; Jha, *et al*, 2018).

Objetivo: Identificar un perfil lipidómico sérico que permita diferenciar entre esteatosis simple y esteatohepatitis no alcohólica en sujetos mexicanos con obesidad.

Metodología: Se incluyeron 375 sujetos con obesidad severa, sometidos a cirugía bariátrica en el Hospital General –Dr. Rub n Leñero". De ellos, 36 fueron controles (sin esteatosis), 78 con esteatosis, 122 EHNA limítrofe y 139 con EHNA. El perfil lipidómico circulante se determinó mediante espectrometría de masas.

Resultados: Se identificaron 31 clases de lípidos, incluyendo glicerolípidos, glicerofosfolípidos, esfingolípidos y colesterol; 12 de ellos (principalmente glicerolípidos y glicerofosfolípidos) presentaron diferencias significativas al comparar controles, esteatosis y EHNA. Aunque los triacilglicéridos presentaron un aumento gradual y significativo al comparar entre los distintos grupos de estudio, éstos no logran diferenciar la esteatosis y la EHNA. En contraste, la concentración de plasmalógenos de fosfatidilcolina se encontró significativamente disminuida en sujetos con esteatohepatitis comparado con sujetos con esteatosis (p=0.004). Además, la concentración de plasmalógenos correlacionó negativa y significativamente con esteatosis, inflamación, balonamiento, puntaje de NAS, transaminasas, niveles de glucosa y presentó una correlación positiva con la adiponectina sérica (p<0.05). Estos resultados son consistentes con lo observado en modelos animales, los cuales sugieren que los plasmalógenos protegen contra el daño producido por estrés oxidante y las lesiones hepáticas inducidas por lípidos tóxicos como el colesterol libre (*Eun Jang* et al 2017).

Conclusiones: El presente estudio confirma que los triacilglicéridos son los principales lípidos circulantes que se incrementan en el hígado en presencia de HGNA; sin embargo, las concentraciones plasmálogeno de PC circulante parece ser un buen candidato evaluar la progresión de esteatosis a esteatohepatitis.

Phylogenetic characterization of the genus *Tobamovirus* in children younger than six months of age

Yarenci Aguado[1], Blanca Taboada[1], Pavel Isa[1], Susana López[1], Angélica Serrano[2], Patricia Morán[2], Liliana Rojas[2], Horacio Pérez [2], Javier Torres[3], Cecilia Ximenez[2], Carlos Arias[1].
[1]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Av. Universidad #2001, Col. Chamilpa C.P. 62210. Cuernavaca, Morelos, México. [2] Unidad de Medicina Experimental, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México, México. [3] Unidad de Investigación Médica en Inmunología, Hospital de Pediatría, Centro Médico Nacional Siglo XXI, IMSS, Ciudad de México, México.
arias@ibt.unam.mx. Tel. (777) 329-1661

Plant viruses are frequently found in the gastrointestinal tract of adults. Nevertheless, they have been rarely identified in children younger than one year (Zhang T, 2005) and there is only one study that has reported the presence of plant viruses in a 6 moth old child. Viruses in the *Virgaviridae* family (positive sense, single-stranded RNA) are the most commonly reported in adults. This family comprises 7 different genera and 59 species. *Tobamovirus* is the largest genus, having 37 species that infect many economically important vegetables, including pepper, tomato, tobacco and potato, among others.

We currently carrying out a study, in the town of Xoxocotla, Morelos, aimed to characterize the gastrointestinal virome of children at an early stage of life. So far, we have analyzed, by NGS sequencing, monthly fecal samples from three children up to six months of age. Interestingly, we found thirteen different species of the *Tobamovirus* genus in these samples and were able of assemble contigs larger than 500 pb from six of these species that were used to obtain the complete genome sequence of several viruses: i) four of tropical soda apple mosaic virus (TSAMV); ii) three of the pepper mild mottle virus (PMMoV) and; iii) one of tomato mosaic virus (ToMV). In order to characterize these new *Tobamovirus* genomes, a phylogenetic analysis was done using the 340 complete genome sequences of all reported *Tobamovirus* species in Genbank. In the phylogenetic tree, it can be observed eight different clades that have been reported to infect eight different families of plants. The contigs and genomes characterized segregated, as expected, with their own species, localized in two clades of different plant families (*Solanaceae* and *Cactaceae*), which are common in Mexico. It is important to point out that there are few complete genomes of *Tobamovirus* species in Genbank; for example, of TSAMV and PMMoV viruses there are only 2 and 18 complete genomes, respectively, and we were able to assemble four more of TSAMV and three form PMMoV. The *v*iruses found in this work were identified commonly in samples from children even at 15 days after birth. Our hypothesis is that these viruses were transmitted to children by their mothers, since the same virus species were also identified in the mothers‘ samples (unique samples taken at the beginning of the study).

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Isolation and purification of *E.coli* DH5α bacteriophages in wastewater and river water samples from Nuevo Leon, Mexico.

Anaí Amaral García, Carlos Eduardo Hernández Luna, Licet Villarreal Treviño y Juan Francisco Contreras Cordero.
Universidad Autónoma de Nuevo León. Pedro de alba S/N, Ciudad Universitaria, San Nicolás de los Garza, Nuevo León. CP 66455. +528183294000 Ext.6432. Email: anai.amaral96@gmail.com

Introduction: The bacteriophages are ubiquitous, they have been found in various environments such as soil, bodies of water, fecal matter, wastewater, etc. They are infectious entities, as their name indicates they infect bacteria and generally provoke the lysis of their hosts. The infectious characteristics that these viruses possess attract interest and nowadays they are being applied for detection and control of bacteria. Despite the importance and abundance of these entities, their study has been very limited and most of them are still unknown.

Objective: To determine the presence and variability of bacteriophages that infect strains of *E. coli* DH5α in wastewater and river water from Nuevo León, Mexico.

Materials, and methods: The samples used for this study were collected from wastewater and river water in the state of Nuevo Leon. The samples were subjected to an enrichment process to potentiate the development of the present bacteriophages and isolate a variety of them from each sample source. After getting a positive result in each type of sample, we purified them to obtain pure strains. We characterized each strain according to their lytic plaques. After achieving enough concentration, we proceed to extract the genetic material for the bacteriophages and performed a restriction assay.

Results: We observe a great diversity of lytic plaques ranging from 0.8 to 1.2 mm in diameter, then we obtained one pure strain from each sample and characterized them based on the difference between their lytic growth. In addition, we obtained a viral titer greater than $1x10^9$ pfu/mL used to extract a large amount of genetic material, this was characterized with restriction patterns generated by the enzymes *EcoRI*, *XbaI* and *BamHI*.

Conclusions: The host *E. coli* DH5α represent a good model for the isolation of phages from different water samples.

Participation of the α-1 and α-2 helixes in the specificity of the toxin Cyt1Aa from *Bacillus thuringiensis* subsp. *Israelensis*

Paulina Anaya Cárdenas[1], Mary Carmen Torres Quintero[1], Janette Onofre Lemus[1], Alejandra Bravo de la Parra[1], Mario Soberón Chávez[1]
1 Instituto de Biotecnología. Universidad Nacional Autónoma de México. Departamento de Microbiología Molecular. Av. Universidad 2001, Chamilpa, 62210 Cuernavaca, Morelos Tel. +52 (777) 329-1624 Correo: mario@ibt.unam.mx

Key Words: biocontrol, *Bacillus thuringiensis*, toxins, specificity

Abstract

Bacillus thuringiensis is a gram positive bacteria that produce parasporal crystalline structures composed of insecticidal proteins. These proteins, known as Cry and Cyt toxins are pore forming proteins that have toxicity against different insect orders.

Cry toxins are toxic to Coleoptera, Lepidoptera and Diptera. On the contrary, Cyt toxins are Dipteran specific, killing mosquitoes and black flies. Cyt toxins also show cytotoxic activity against a variety of insect and mammalian cells, including erythrocytes, lymphocytes, and fibroblasts.

Bacillus thuringiensis subsp *israelensis* (Bti) produces four 3d-Cry proteins, Cry4Aa, Cry4Ba, Cry10Aa and Cry11Aa, along with two Cyt proteins, Cyt1Aa and Cyt2Ba. Bti has been used worldwide for the control of different mosquito and black fly species that are vectors of important human diseases.

The mode of action of Cyt toxins begins with the ingestion of the crystals by the larvae which are solubilized in the midgut juice of susceptible insects and then activated by gut proteases. The activated toxins undergo structural changes to insert into the membrane and form oligomeric pores that induce cell lysis. In contrast to Cry toxins, Cyt toxins do not bind insect gut protein molecules and directly interact with the cell membranes.

Cyt toxins are structured as a single α-β domain composed of two outer layers of α helix hairpins wrapped around a β-sheet. Previous work showed that single point mutations in helix α-1 were involved in the specificity of Cyt1Aa toxin as the hemolytic and insecticidal activities were separated establishing a differential recognition of the cell type.

To determine the role of helixes α-1 and α-2 in Cyt1Aa in the specificity of this toxin, single point mutations in these helixes were characterized. We will present data showing the insecticidal and hemolytic activities of these Cyt1Aa variants and the analysis of binding to *Aedes aegypti* brush border membranes and to red blood cells. Our data suggests that Cyt1Aa helixes α-1 and α-2 have a fundamental role in binding and specificity to mosquito and red blood cells.

The genetic variability of E7 HPV16 oncoprotein in cervical samples of woman from State of Guerrero.

Ramón Antaño Arias, Jorge Adán Navor Hernández, Julio Ortiz Ortiz, Marco Antonio Jiménez López, Luz del Carmen Alarcón Romero, Dinorah Leyva Illades, Oscar del Moral Hernández y Berenice Illades Aguiar.
Laboratorio de Biomedicina Molecular, Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Guerrero. Avenida Lázaro Cárdenas S/N, Ciudad Universitaria, Chilpancingo, Gro, México. CP 39090. Tel (747) 4710901. Email. b.illadesaguiar@gmail.com

Background: The E7 protein of Human Papillomavirus 16 is a oncoprotein related to papillomaviruses oncogenicity. The expression of E7 and E6 in cervix are necessary but no sufficient to develop cancer. E7 bind to tumor suppressor protein retinoblastoma (pRB), the binding release E2F that activate transcription of genes for cell cycle progression. Some mutations in the gene E7 have been reported worldwide and a recent study with E7 genetic variants show increased affinity to pRB and transforming capacity due a new phospho acceptor site. Due the role of the E7 oncoprotein in oncogenicity is imperative to know the genetic variability and its effect in tumor development. However, no studies have been made in México to analyze mutations in the E7 gene.

Objective: To analyze the genetic variability of E7 HPV16 and its association with cervical lesions and cancer.

Methods: 190 samples of biobank of Biomedicine Molecular Laboratory of University Autonomous of Guerrero was analyzed by sequencing the E7 gene and aligned with HPV16 reference sequence NC_001526. All cervical samples had infection with HPV16.

Results: E7 HPV16 had 6 genetic variants in the population studied. Only two genetic variant had missense mutations E7-A712 (H51N) and E7-G647 (N29S). The most frequent genetic variant in all samples and samples with cervical cancer was E7-C732/C789/G795.

Conclusion: Our result shows different genetic variability of E7 HPV16 compared with studies in others population of study. The E7 genetic variant E7-C732/C789/G795 has increased risk for cervical cancer as show the OR analysis.

Marine bacterial diversity with high potential to degrade pyrene isolated from Rosarito in Baja California

Cynthia Lizzeth Araujo-Palomares[1], José Vinicio Macías-Zamora[1], Nancy RamírezÁlvarez[1], Bianey García-Lara2, Alma Rosa Corrales-Escobosa2, Hortencia Silva-Jiménez[1*]
[1]Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California, Carretera Tijuana-Ensenada, No. 3917, Fraccionamiento Playitas, 22860, Ensenada, Baja California, México. Phone +52646-1745462 Ext. 64717.
1*silvah@uabc.edu.mx
[2]Departamento de Química, Universidad de Guanajuato, Lascuráin de Retana 5, 36000 Guanajuato, México.

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are recalcitrant and persistent compounds in the environment. The US Environmental Protection Agency (EPA) have catalogued 16 PAHs as priority contaminants by their toxicity and mutagenic and/or carcinogenic effects. PAHs in marine environments are likely to increase due to anthropogenic activities in the coastal zones, such as sewage discharges, marine traffic of ships, loading and unloading of fuels, and these represents a longterm environmental risk. For instance, considerable amounts of PAHs, including high molecular weight (HMW) like pyrene, have been reported in marine sediments of several points of the Coast of Baja California such as Todos Santos Bay and Rosarito Port area in Baja California, México.

An alternative to remove PAHs of the environment is using hydrocarbonoclastic bacteria. Therefore, the aim of this work was to study the bacterial diversity of indigenous isolates with potential to degrade pyrene. We used MALDI-Biotyper (Bruker Daltonics) as a powerful analytical tool for identification. Bacteria were isolated from surface seawater and marine sediments samples of three sites from the coast of Rosarito Port, B.C., Mexico.

Total concentration of PAHs in collected samples was quantified by GC-MS, showed values ranged 0.461-0.525 ng mL^{-1} and of 74 -266 ng g^{-1} in seawater and sediments samples, respectively. A total of 52 bacteria with capacity to grow in 25 mg L^{-1} pyrene as sole carbon and energy source were taxonomically identified and classified by MALDI-Biotyper system by comparing the mass spectra with library and/or to use chemometric tools to evaluate possible differences to isolate level.

The identified isolates were represented by three phylogenetic groups: Firmicutes, Actinobacteria and Proteobacteria.

Some of these isolates appear to be excellent candidates to continue PAHs degradation studies.

Keywords: PAHs, marine bacteria, MALDI-TOF MS.

Functional analysis of *Rep* and *CP* promoters of a natural mutant of Tomato yellow leaf curl virus (TYLCV)

Jesús Aarón Avalos-Calleros.[*1], Guillermo Pastor-Palacios.[2], Bernardo Bañuelos Hernández.[3], Salvador Ambriz-Granados.[1], Gerardo Rafael Argüello-Astorga.[1*]
[1]División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica, Camino a la Presa San José, 78216 San Luís Potosí, San Luís Potosí, México. [2] CONACYT-IPICYT Consorcio deinvestigación, innovación y desarrollo para las zonas áridas, [3] Facultad de Agronomía y Veterinaria, Universidad La Salle, Av. Universidad 602, Col. Lomas del Campestre, C.P. 37150 León, Gto, México.
*Corresponding authors: jesus.avalos@ipicyt.edu.mx; grarguel@ipicyt.edu.mx.

Tomato yellow leaf curl virus (TYLCV), a monopartite begomovirus native to the Middle Eastern-Mediterranean region, is a major threat to tomato production worldwide. TYLCV was introduced to Mexico circa 1996. In 2011, a variant of this exotic virus was isolated from two plants collected in the locality of Aquismón, San Luis Potosi. In *Solanum pimpinellifolium* was found coinfecting with the native begomovirus ToChLPV, whereas in a *Solanum lycopersicum* plant was found coinfecting with ToSLCV. This new variant, designated TYLCV-[SLP], differs from others described to date for two notorious changes in the viral intergenic region: a deletion of 29 bp upstream of the conserved ~~s~~tem-loop" element of the replication origin (*Ori*), and a duplication of a 42 bp segment downstream the latter element. This duplicated segment included a Conserved Late Element (CLE) which is responsive to the begomovirus transactivator, TrAP. These natural mutations may have altered the functional properties of the promoters of two divergent genes and eventually, the pathogenic characteristics of TYLCV. We functionally analyze the promoters of the genes encoding Rep and CP proteins of TYLCV-[SLP] and the wild type TYLCV (represented by an isolate from Sinaloa). The viral promoters were fused to the GUS reporter gene and analyzed by transient expression assays in tobacco protoplasts and in *Nicotiana benthamiana* plants. The experimental results of the protoplast assays revealed that the activity of TYLCV-[SLP] Rep promoter is very weak, ≥15 times lower than the homologous promoter of TYLCV wt. A similar pattern was observed in transitory expression assays *in planta* (agroinfiltration), although the difference was not as dramatic as in the protoplast experiments: the promoter of TYLCV-SLP turned out to be ~4 times weaker than its WT counterpart. These observations suggest that the 29-bp segment that was deleted in TYLCV-[SLP] contains one or more cis-elements that activate the *Rep* gene transcription. A comparative analysis revealed the existence of an 18-bp sequence in the former region which is conserved in most begomoviruses native to the Old World but is absent in all New World begomoviruses. This conserved sequence, which we dubbed ~~i~~teron-associated conserved element" (**ItACE**), could be a potent regulatory element of Rep gene expression. In contrast, the CP promoters of TYLCV-[SLP] y TYLCV-[Sin] displayed a similar transcriptional activity in absence of viral factors. Because of the duplicated 42-bp segment containing a CLE, we presumed that the activity of TYLCV-(SLP) CP promoter may be higher in the presence of the TrAP protein.

The role of microRNA-927 during the persistent infection with DENV serotype 2 in C6/36 mosquito cells

Avila-Bonilla R. G., Salas-Benito J. S.
Doctorado en Ciencias en Biotecnología, Escuela Nacional de Medicina y Homeopatía, Instituto Politécnico Nacional (ENMyH-IPN) Guillermo Massieu Helguera 249, La Escalera-Ticomán, Mexico City, CP 07320, Mexico.
Tel:5557296000 Ext: 55336. E-mail: avilab.r.g@gmail.com
Keywords: Dengue virus, persistent infection, miRNA

Abstract
Dengue virus (DENV) is the major emerging arbovirus (arthropod-borne virus) transmitted by *Aedes* mosquitoes which is prevalent in the tropical and tempered areas worldwide. In mosquito cells DENV can remain for a long period of time establishing a persistent infection without cytopathic effect (CPE)[1] that requires a balance between virus replication and antiviral host response. Recent evidence demonstrates that microRNAs (miRNAs) can affect the virus replication and pathogenesis either by post-transcriptional inhibition of the host-genes or through direct binding to the virus genome[2]. Mosquito cell lines have been used previously to study arboviruses persistent infections because they are easy to manage and several variables can be controlled[1]. Recently, using deep sequencing we found that miR-927 is overexpressed in C6/36 mosquito cells persistently infected with DENV serotype 2 suggesting its participation3 but the exact role is not complete understood. In this work we inquired the role of miR-927 in C6/36 persistently infected with DENV2 during 30, 40 and 57 weeks (C6L-30, 40 and 57). Using RTqPCR we found that miR-927 was up regulated only in C6L-57 cells. Target prediction of miR-927 using RNAhybrid and RNA22, implies genes related in posttranslation modification (SUMO), translations factors (eIF-2B) immune innate system (NKIRAS), exocytosis (EXOC-2) endocytosis (APM1) and cytoskeleton (FLN-B). All genes were characterized by RT-qPCR in C6-L57 cells. The EXOC-2 and APM1 genes were down-regulated in C6-L57 cells compared with acutely infected cells (MOI 0.1) indicated their possible regulation by miR-927.
Additionally, the transfection with miR-927 inhibitor display changes in the expression of eIF-2B, EXOC-2, APM1, NKIRAS and FLN-B demonstrating a regulation of miR-927 over the target genes. Interestingly, when the miR-927 inhibitor was used, DENV2 shown low levels of intracellular genome copy numbers but higher in the supernatant suggesting that miR-927 might be important in the morphogenesis of DENV2 in persistently infected mosquito cells.

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The *tyrR* gene encoding the transcriptional regulator TyrR from *Azospirillum brasilense* Sp7 is involved in catabolism of alanine.

Jijón Moreno Saúl, Xiqui Vázquez María Luisa, Castro Fernández D. Carolina, Baca Beatriz E. and Ramírez-Mata A.*
Centro de Investigaciones en Ciencias Microbiológicas, Benemérita Universidad Autónoma de Puebla. Edif. IC11, Ciudad Universitaria, Puebla Pue. CP72570 Puebla México. beatriz.baca@correo.buap.mx

Azospirillum brasilense is one of the most studied and isolated bacterium from a large diversity of agronomic plants, and in wide world. The benefits to plants by inoculation with *Azospirillum* have been primarily attributed to its capacity to fix atmospheric nitrogen, but also to its capacity to synthesize phytohormones, in particular indole-3-acetic acid (IAA). The principal pathway synthetizing IAA involved the indole pyruvic acid intermediary in both *A. brasilense* and *Enterobacter cloacae*, is the pathway involved the *ipdC* gene, which in *E. cloacae* is regulated by the transcriptional regulator TyrR. In this study a *tyrR*-deficient derivative of *A. brasilense* Sp7 named *A. brasilense* 2116 was constructed and a plasmid carried out the *tyrR* gene transferred to 2116 strain, to complement the mutation. The data obtained indicate that transcriptional regulation by TyrR is responsible of utilization of amino acid alanine as carbon source and partially as nitrogen source, but is not involved in IAA biosynthesis. The comparison of its deduced amino acid sequence and structural analysis between TyrR domains proteins indicates that TyrR contains in the N-terminus a PAS domain (**P**er-**A**RNT-**S**IM, residues from 79 to 146), and an ACT domain (**A**spartate kinase, **C**horismate mutase and **T**yrA), containing a highly conserved aspartate (D) and arginine (R) amino acid residues, which both of them has been implicated in binding to aromatic amino acids and showed to participate in oligomerization of protein and module activation or repression of genes. Following by a central domain that contains a putative ATP binding site identified on the basis of sequence homology with the ATP binding sites Walker A (GXXGXGKE) and B (TVFXDE), this domain is responsible for ATP hydrolysis; it is indispensable and often sufficient to activate σ54-dependent transcription and revealing that TyrR protein exhibits significant homology to those of other regulators belong to a bacterial superfamily of AAA^{+} proteins, known as bacterial enhancer-binding proteins (bEBP), such as NtrC (Rippe et al. 1998), FleQ, (Matsuyama et al. 2015), and also with TyrR protein from *E. coli* and *E. cloaceae* (Ryu and Patten, 2008). However, TyrR from *A. brasilense* completely differs in respect that it regulates transcription likely by the σ54-dependent promoters as determined in *P. aeruginosa* (Sarwar et al., 2016), because of the highly conserved loops—L1, which contains the signature GAFTGA that is essential for contact with the σ54 subunit. The structure analysis of TyrR protein revealed three putative domain-binding sites for aromatic amino acids and ATP cofactors, as well to bind DNA sequences of promoter-operator regions. The promoter sequences encompass one to three TyrR boxes, which were found in genes predicted to be regulated by TyrR. The data suggested that TyrR protein plays a major role in the regulation of genes that are essential for the transport, and degradation of aromatic amino acids.

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Characterization of the response of *Arabidopsis thaliana* in interaction with auxin-producing rhizobacteria isolated from Chihuahuan Desert plants.

Fernando Balderas Hernández [1], Claudia Navarro Múñoz[1] Jorge Sáenz-Mata[1] Rubén Palacio-Rodríguez[1] José López-Bucio[2], Juan Ángel Ayala Rodríguez[2]
[1] Facultad de Ciencias Biológicas, Universidad Juárez del Estado de Durango, Av. Universidad S/N, Fracc. Filadelfia, C.P. 35010 Gómez Palacio, Durango, México
[2] Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Edificio B3, Ciudad Universitaria, C.P. 58030 Morelia, Michoacán, México

Key words: Rhizobacteria, Auxin, PGPR, *Arabidopsis thaliana*

The bacteria that inhabit the rhizosphere of plants has the ability to stimulate the growth of the plant there fore they have been denominated as Plant Growth Promoting Rhizobacteria(PGPR). They have already been described several of the mechanisms that these bacteria use to stimulate growth of the plant such as the capacity of synthesizing phytohormone auxins, phosphate solubilization and ACC deaminase production. Auxin acts as a general coordinator of plant growth and development, transferring information over both long and short ranges. Auxin famously appears to be extraordinarily multifunctional, with different cells responding very differently to changes in auxin levels. The interactions of rhizobacterias promoting auxin production with the model organism *Arabidopsis thaliana* provide information that helps us understand the relationship of these bacteria with their host plant. The isolation of the rhizobacteria was done from the endo and ectorizosphere of 7 plants of the Chihuahuan Desert in three different culture media Luria Bertani (LB), King B (KB) and NFB in which it was possible to isolate 180 strains, once isolated the bacteria were put into interactions in direct contact with the auxin-signaling reporter *DR5::β -glucuronidase* (GUS) in order to identify the strains with auxinic response. Of the 180 strains isolated 6 showed an auxinic response, the primordia were recorded in different stages of the formation of lateral roots to the seedlings that had interaction with these strains, these 6 strains were again put in direct contact with the cell-cycle marker *CycB1:uidA* and mutants defective in auxin signaling (*tir1/afb1/afb2* [receptors], *slr1* [repressor] and *arf7/arf19* [transcription factors]) and *A. thaliana Col 0* as control. The 6 strains significantly inhibited the growth of the main root and shoot but most promoted the formation of lateral roots. Once the interactions were completed, the strains were identified by sequencing the 16s rRNA region

Characterization of a putative component of the Fla 2 flagellar system of *Rhodobacter sphaeroides**

Teresa Ballado[1], Javier de la Mora[1], Clelia Domenzain[2], Laura Camarena[2], Georges Dreyfus[1]
Instituto de Fisiología Celular[1] and Instituto de Investigaciones Biomédicas[2]
Universidad Nacional Autónoma de México. Circuito Exterior s/n, Coyoacán C.P. 04510, México City. Tel. 5255-5622-5618. gdreyfus@ifc.unam.mx

Rhodobacter sphaeroides is a purple non-sulfur photosynthetic α-proteobacterium that is metabolically diverse, grows either aerobically or anaerobically and, fixes nitrogen. This bacterium posses two complete flagellar systems. The Fla1 system that expresses a single subpolar flagellum, acquired by horizontal transfer from an ancestral γ-proteobacterium and is expressed constitutively under routine laboratory conditions. The products encoded by the *fla2* gene set produces several polar flagella. However, the expression of these genes is only achieved under very particular conditions. Fla2 flagella were detected in a mutant strain lacking the master activator of the *fla1* genes that acquired a gain of function mutation in the histidine kinase CckA. A bioinformatic analysis of the *fla 2* gene cluster revealed several open reading frames (ORF'S) of unknown function.

In this work we characterize RSWS8N_12050. This orf is part of an operon with the following gene arrangement: *fliL2*-RSWS8N_12050-RSWS8N_12055- *motA2-motK*.
RSWS8N_12050 was mutated and the swimming behavior was analyzed on swimming soft agar plates. A reduction of the swimming diameter was observed when compared to the wild type strain AM1. This result suggested that RSWS8N_12050 is involved possibly in the performance of flagellar assembly

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DETERMINATION OF THE REGULATORY NETWORK OF QUORUM SENSING IN *Rhizobium leguminosarum biovar viciae 248*

Lorena Y. Balón-Rosas[1], Ángeles Pérez-Oseguera[1], Miguel A. Cevallos-Gaos[1].
Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México. Av. Universidad s/n, Col. Chamilpa C.P. 62210. Cuernavaca, Morelos, México. Tel. 01 7773291690. Correo: yamileth@ccg.unam.mx

Quorum Sensing (QS) is a mechanism of communication between bacteria that depends on cell density estimate to act in a concerted manner. QS is a mechanism by which bacteria regulate gene expression in accordance with population density through the use of diffusible signaling molecules, known as autoinducers. In *Rhizobium leguminosarum biovar viciae*, the QS involves N-acyl homoserine lactone synthase (LuxI) and a LuxR-type transcriptional regulator. Genes encoding LuxI/R proteins almost always are side by side. However, analyses of bacterial genomes have shown the existence of genes similar to the canonical *luxR*-type which are not associated to a luxI gene. These LuxR homologous are known as orphans or ~~-s~~olos".

In this work, we were used as a model *Rhizobium leguminosarum bv. viciae 248* strain, whose sequence is known. In this genome, we identified 3 luxI/luxR canonical systems: CinI/CinR (chromosome); TraI/TraR and RhlI/RhlR (plasmid) and 13 LuxR-type orphans. We also identified the binding domains to an AHL in the N-terminal region of each LuxR-type protein. The regulatory hierarchy of QS systems in this bacterium were determined through site-directed mutagenesis & AHL detection assays, and our data allow us to propose that the CinI/R chromosomal is the system with higher hierarchy, because insertions in CinI synthase is inhibited production of its AHL and the other two systems (lesser hierarchy). In contrast, when we mutated traI or rhlI synthase, production of AHL in CinI/R system remains intact. To assess their hierarchy in the regulatory network, transcriptional fusions were builted with the promoters of the luxI/R genes and basal levels of expression were observed in our mutants of lesser hierarchy because they depend on the chromosomal QS system.

With the AHL analyses throughTLC we were able to identify which AHL produce each LuxI synthase. Also with a collection AHL chemically synthesized we were capable to identify the chemical nature of each one of the AHLs produced by *Rhizobium leguminosarum biovar viciae 248.*

Stress study in *Bacillus subtilis* by use of artificial ribo-regulators

Diana Barceló-Antemate [1*], Zuemy Rodriguez-Escamilla [2] and Enrique Merino-Pérez[2+]
[1]*Universidad Autónoma del Estado de Morelos,*
[2] *Instituto de Biotecnología-UNAM. Departamento de Microbiología Molecular*
**dbarcelo@ibt.unam.mx; +merino@ibt.unam.mx*
Avenida Universidad 2001 Col. Chamilpa. Cuernavaca 62210 Cuernavaca, Mor. México.
Tel: +527773291629.

Bacteria spend most of their lives under constant stress. The ability to survive under restrictive growth conditions requires special strategies, which are of fundamental significance for microbial life in natural ecosystems[1]. The main strategies used by microorganisms are general stress proteins, alternative sigma factors and small RNAs (sRNA). The latter are crucial transcriptional regulatory elements in the bacterial response to stress that commonly act inhibiting the translation initiation process by its binding to the Shine-Dalgarno sequence of their corresponding mRNA targets[2].

With the arrival of synthetic biology, artificial regulatory sRNA, called ribo-regulators, have been designed, based on the properties of natural bacterial sRNAs, to modulate the expression of basically any gene of interest[3].

The aim of this project is to identify regulatory networks for stress response in *B. subtillis*, which transiently inhibits, through the use of artificial sRNAs, the expression of essential genes, *dnaN, sigA and pheS,* involved in the processes of replication, transcription and translation, respectively. The study of adaptive network that reflects the dialogue between *Bacillus* and the limits of growth is very important for the understanding of the general physiology of Bacillus. Such information will be of universal value and may be generalizable to other cultivable bacteria and superior single-cell organisms. The evaluation of regulation efficiency for ribo-regulators will be performed through the specific quantification of target genes with the help of reporter genes.

In conclusion, our research proposal that uses ribo-regulators targeting essential genes will allow the study of stress response in conditions never seen before.

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Study of the regulation of PHB depolymerization in *Azotobacter vinelandii*

Thalía Barrientos Millán, Libertad Alejandra Adaya García, Josefina Guzmán Aparicio, Soledad Moreno León, Carlos Peña Malacara, Guadalupe Espín Ocampo, Daniel Segura González
Instituto de Biotecnología. Universidad Nacional Autónoma de México.
Av. Universidad 2001, Chamilpa, CP 62210, Cuernavaca, Morelos.
Tel. 7773291629 Ext. 27629
thaliabm@ibt.unam.mx, daniel@ibt.unam.mx

Polyhydroxyalkanoates (PHA) are polyesters produced by various archaea and bacteria as reserve of carbon, energy and reducing power. These polymers are intracellulary accumulated under conditions of carbon source excess and nitrogen, phosphorous or oxygen limitation and are mobilized (utilized) when the carbon source is scarce. *Azotobacter vinelandii* is one of the bacteria capable of producing PHA, it synthesizes mainly polyhydroxybutyrate (PHB) and the importance of these compounds in industry is that they can be used to manufacture biodegradable plastics to replace petroleum-based plastics. PHB synthesis in *A. vinelandii* starts from two molecules of acetyl Co-A, through three enzymatic steps catalyzed by β-ketothiolase, acetyl Co-A reductase and PHB synthase, encoded by the *phbA, phbB* and *phbC* genes, respectively; while PHB degradation (mobilization) is carried out by the enzymes PHB depolymerases, hydroxybutyrate dehydrogenase, succinyl Co-A transferase and, again, β-ketothiolase. Although these two processes occur simultaneously, giving rise to a constant synthesis/mobilization cycle, some mechanism is needed to control the balance of this cycle, thus favoring synthesis or degradation depending on the metabolic conditions.Some regulators of the PHB biosynthetic genes are known in *A. vinelandii* but nothing is known about the control of PHB degradation, it is possible that both processes would be controlled by the same regulation systems.
In this work, we show that PhbF is one of the regulators involved in biosynthesis process, that acts repressing the expression of *phbP1* gene that encodes a phasin, a granuleassociated protein that promotes granule formation only in presence of PHB. In *A. vinelandii*, several genes encoding possible PHB depolymerases have been found, one of them, *phbZ1*, shares its regulatory region with *phbP2* gene (involved in biosynthesis) and a possible PhbF binding site is found in the regulatory region shared by these two genes.In this study, the participation of PhbF regulator in the control of mobilization process will be demostrated by the analysis of *phbZ1* gene expression in both UW136 and UW136*phbF* strains through qRT-PCR and *phbZ1-gusA* transcriptional fusions, as well as the analysis of their PHB accumulation phenotypes.

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Characterization of chaperonine HpGroEL of *Helicobacter pylori*

Selene Becerril-Huesca[1], Cristhian Sánchez-Cruz[1], Laura Isabel Vázquez-Carrillo[1], Marco Antonio González-López[2], José de Jesús Olivares-Trejo[1*].

[1*]Posgrado en Ciencias Genómicas, Universidad Autónoma de la Ciudad de México, San Lorenzo 290, Col. Del Valle, C.P. 03100, Ciudad de México, México. *e-mail: olivarestrejo@yahoo.com. Tel: +525554886661 Ext. 15309.
[2] Cátedra CONACYT-Instituto Nacional de Cancerología. Av. San Fernando No. 22, Col. Sección 16, C.P. 14080. Ciudad de México, México.
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Helicobacter pylori is a human gram-negative pathogen that invades the human gastric epithelium. The infection caused by this bacterium can generate peptic ulcer and develop stomach cancer. This pathogen needs iron to survive into the human host and it can support its cellular growth using Hb (human haemoglobin), haem or Tf (transferrin) as the only iron source. To obtain iron from these sources this bacterium expresses proteins such as FrpB1, FrpB2 or FrpB3. HpGroEL is another protein that is involved in iron acquisition. This protein can bind iron attached to tetrapyrrolic ring. We believe that this characteristic is own of *H. pylori* because the GroEL of *E. coli* does not have. In addition, *H. pylori* secretes the chaperonin while *E. coli* not. Unfortunately, we do not know if *H. pylori* has chaperonin activity and whether it is regulated under several iron sources. For this reason, in this work both characteristics were investigated. We observed that *H. pylori* secreted HpGroEL, even if the iron source was changed. Additionally, the amount of chaperonin secreted was greater than that of total proteins. The folding capability also was tested for HpGroEL, interestingly HpGroEL showed folding activity to pH acid or alkaline, however a control with chaperonin from *E.coli* protein was unable to fold at the same pHs. Our overall results indicate that HpGroEL is secreted to the culture media, it binds iron and also it is capable of folding under several pHs. We think that these characteristics allow *H. pylori* to support to the extreme conditions present in the human stomach in order to establish the infection process.

Influence of foreign bus passengers in the composition of the microbiome of Mexico City subway

Ana Luisa Bravo, Mariana Peimbert
ana.sahuaripa@gmail.com
Universidad Autónoma Metropolitana Unidad Cuajimalpa
Avenida Vasco de Quiroga 4871, Col. Santa Fe Cuajimalpa.
C.P. 05348, Ciudad de México

Abstract

Urban microorganisms can be defined as a vast and diverse sets of resident and transient microorganisms that occur in or within the numerous habitats that make up urban systems[1]. The interest in the characterization of the microbiome in urban environments is based on the importance of understanding the associations between occupant health and microbial exposure in-built environments. Some bacteria of the human microbiome contribute beneficially to health in different ways[2]. Exposure to a greater diversity of microorganisms may confer protection against the onset of respiratory conditions[3] while the reduction of microbial diversity in closed environments has been related to the development of adverse respiratory conditions such as asthma[4].

Humans interact and acquire bacteria and other organisms depending on the environments through which they transit, the surfaces they touch and the dynamics of the environment in the cities. Bacteria colonize humans and they can function as microbial vectors that shape the microbiome of the surfaces they come in contact with[5]. In addition to desquamation, microorganisms associated with the skin can be transferred to surfaces and floors after physical contact. The dust that has settled on floors carpets is rich in microorganisms, because it is a complex mixture of inorganic and organic particles, probably represents an integrating record of microbial biodiversity in occupied spaces[6].

The aim of this study was to evaluate the transference of microorganisms through bus passengers from different regions of Mexico and their dispersion in the microbiome of the Mexico City subway through the massive sequencing of the 16S ribosomal gene. The microbial diversity of floors and surfaces of the arrival platforms and corridors at bus terminals were compared to those obtained on the floors and surfaces of the entrances and exits of the subway stations near the bus terminals.

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Inhibitory effect of the nanoparticled compound Nbelyax™ over viral and fungal respiratory pahogens.

Carlos Cabello Gutiérrez*, Gabriel Palma Cortés, Fernando Hernández-Sánchez, Luis Angel Pérez-Moreno.
Departamento de Virología y Micología, Instituto Nacional de Enfermedades Respiratorias –Ismael Cosío Villegas". Calzada de Tlalpan 4502, Col. Sección XVI, Deleg. Tlalpan. CDMX. México. C.P. 14080. *carloscginer@gmail.com

It is widely known that the best way to avoid microbial transmitted diseases is by means of preventive actions. For example, hand washing and surface disinfection of exposed surfaces effectively reduce dissemination of respiratory tract bacterial, fungal or viral infections. Available cleaning products advertise their effectiveness on this basis, but usually have no proved anti-mycotic or anti-viral activities and include health risk products as vehicles in their formulae.

Recently developed alternatives based on nanotechnology are using human-safe and biocompatible active principles to avoid health and environmental risks. One interesting approach came from the use of combinations of essential oils from citrus fruits as disinfectant agents; having a demonstrated antibacterial activity without toxic side effects for human tissues.

The main goal of the present work was to assess the *in vitro* inhibitory potential of citrus-oil nanoparticles (Nbelyax™) on the replication of air transmitted respiratory viral and fungal pathogens. Two types of highly prevalent virus were tested in this study: Influenzavirus A (IAV) and Respiratory Sincitial Virus (RSV) as well as the most common agent of respiratory disseminated Mycoses, *Aspergillus fumigatus*. Viral type strains from ATCC were assayed in cultured MDCK and LLMK-2 cell lines for IAV and RSV respectively, using a M.O.I. of 1. Single concentration inhibition was estimated by means of indirect immune fluorescence (IIF), and serial dilutions were used to estimate the minimal inhibitory concentration for both virus using RT-PCR. For fungal growth inhibition, sterile paper filter discs were soaked in nanoparticles at various concentrations. Discs were placed in Agar-Saboureaud plates seeded with *A. fumigatus* conidia to evaluate the size of inhibition halos. Nanoparticles were compared against commercial filter discs (Sensidiscos) containing amphotericin, ketoconazole and amikacin.

Results show that Nanoparticles effectively inhibited viral propagation at low concentration as shown by IIF. Infected cells treated with nanoparticles showed lower copy number of viral genomes compared with controls as seen by RT-PCR assays. Cell viability was unaffected by nanoparticles at the optimal virus inhibitory concentration (formazan reduction assay). Nanoparticles arrest the growth of *A. fumigatus* also at low concentrations, and the inhibitory potential was found to be better than those of amikacin, amphotericin or ketoconazole when used at comparable concentrations.

Conclussion: Citrus based nanoparticles effectively eliminate growth of *A. fumigatus* and replication of IAV and RSV in vitro. This finding opens a wide range of potential applications to decrease the dissemination rate of respiratory pathogens.

Cellular organization of the entomopathogenic fungus *Metarhizium anisopliae*

Olga Alicia Callejas-Negrete and Rosa Reyna Mouriño-Pérez
Departamento de Microbiología. Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE).
Carretera Ensenada Tijuana 3918, Ensenada, B.C., México, C.P. 22860
ocalleja@cicese.mx

Metarhizium anisopliae is an entomopathogenic fungus used for biocontrol of several insect pests, including adult African malaria vectors. Recently, *Metarhizium robertsii* has been reported to be rhizosphere-competent and to stimulate plant root development. While interacting with either insects or plants, the fungus must confront different barriers and toxic compounds, which triggers events of cell differentiation and transport of organelles to achieve homeostasis. In fungal cell polarity is an important process for proper growth and morphogenesis. Microtubular cytoskeleton is important to maintain cell shape and intracellular transport of various organelles, such as vesicles, mitochondria, peroxisomes, endoplasmic reticulum, nuclei, and others. In this work we described for the first time the cellular organization and the involvement of the microtubular cytoskeleton in the intracellular distribution of *M. anisopliae*. Using live-cell imaging we observed that in growing hyphae, the Spitzenkörper is localized in the apical dome and moves following the same direction as the hyphal tip. We also observed that mitochondria accumulate at the subapex, and it is a multinucleated organism. We measured the growth rate of the hyphae (0.45±0.066 µm/min), and the interseptal distance (40 ± 7 µm). We determined the Benomyl sensitivity of the fungus and found that the concentration that inhibited hyphal growth rate by 50% is 2.5 µg ml^{-1}. This concentration will be used in further experiments to assess if microtubules are transporting mitochondria, nuclei, and other organelles in *M. anisopliae*.

Keywords: Metarhizium anisopliae, microtubules, biocontrol, organelles.

SURVEILLANCE OF VENEZUELAN EQUINE ENCEPHALITIS USING CATTLE AS SENTINELS IN THE EASTERN MEXICO-US TRANSBOUNDARY REGION

Castillo-Cervantes, K[1,5], Barrios-García, HB[1], Pérez de León, A[2], Travassos-Da rosa, A[3], Leal, G[3], Weaver, SC[3], Estrada-Franco, JG[4].
[1]FMVZ, UAT. 2USDA-ARS. 3UTMB. 4CBG, IPN. 5FMVZ, UNAM
Autor correspondiente: cer_ka@hotmail.com, joseestradaf@hotmail.com

Venezuelan Equine Encephalitis (VEE) is a reemerging zoonotic arboviral disease, which it is caused by the Venezuelan Equine Encephalitis Virus (VEEV) that is transmitted by several species of mosquitoes in the Americas. VEE causes morbidity and mortality in equines and humans and it is considered a permanent health threat in several countries including Mexico and the USA. VEE is characterized by fever and in some cases, it causes central nervous system disorders and death. VEEV is predominantly transmitted by Culex, Aedes and Psorophora mosquito species. VEEV is maintained in nature in a sylvatic cycle circulating in several mammal hosts. Cattle can be used as sentinel species to monitor VEEV activity and its geographic distribution. The objective of this research project was to conduct a bovine serosurvey in the transboundary region between Northeast Veracruz, Mexico and Southeast Texas, USA, as an indicator of VEEV activity. The actual distribution of VEEV in this region is unknown after the last outbreak in the early 1970s that afflicted equines and humans although recent scientific evidence has documented the circulation of VEEV in other parts of Mexico. Between February and June 2014, 538 blood samples were obtained from cattle 6-12-month-old residing in the Mexico-US transboundary region. Samples were processed for analysis by Hemagglutination Inhibition (HI) and Plaque Reduction Neutralization Test (PRNT) to detect antibodies to VEEV subtype IE. Seropositive samples were identified by HI (n= 58; 10.78%) and by PRNT (n= 105; 19.52%). These findings confirmed the utility of cattle as sentinels for VEEV activity. The evident circulation of VEEV in Northeast Veracruz, Mexico and Southeast Texas highlights the risk for the reemergence of VEE in this transboundary region. This type of science-based knowledge informs binational preparedness plans to mitigate the threat of VEE outbreaks to public and animal health.

In vitro antagonistic activity of bacteria isolated from marine ecosystems against *Vibrio parahaemolyticus*, causal agent of Acute Hepatopancreatic Necrosis Disease (AHPND) in *Litopenaeus vannamei* cultures.

Gabriel Enrique Cázares Jaramillo, Zinnia Judith Molina Garza, José Cuauhtémoc Ibarra Gámez, Lucio Galaviz Silva.
Universidad Autónoma de Nuevo León. Facultad de Ciencias Biológicas, Ave Universidad s/n, Ciudad Universitaria, San Nicolás De los Garza, Nuevo León, C.P. 66455. Email: gabrielcazaresj@gmail.com Cell phone: (614) 125-45-28.

Introduction: Aquaculture has as one of the main productions the cultivation of white shrimp (*Litopenaeus vannamei*) worldwide; however, shrimp farming is affected by diseases like the most recent known as Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Disease (AHPND) which is caused by *Vibrio parahaemolyticus*. An alternative method to control the etiological agent of AHPND is the application of probiotics, which, when consumed by the shrimp, give it a defense mechanism against this bacterium, in this way economic losses of production could be reduced.

Objective: Isolate and characterize antagonistic bacteria of marine ecosystems against the pathogenic strain of *V. parahaemolyticus* AHPND+.

Materials and methods: Marine organisms (clam, crab, snail, algae), water and sediments were collected in two locations in the state of Sonora, Mexico. Various samples were processed, and serial dilutions were prepared, which were spread on different plates with TCBS agar, Marine Agar and TSA with 2% NaCl. Different colonies of microorganisms were isolated in the respective culture media. Antagonistic evaluation of bacteria to be challenged was carried out by longitudinal spread in Mueller-Hinton Agar with 2% NaCl and incubated for 24 hours at 30-32°C. Antagonistic effect of the microorganisms to be challenged was evaluated by measuring the zones of inhibition in millimeters.

Results: The different isolated bacteria showed a strong antagonistic activity against *V. parahaemolyticus* and certain bacteria showed partial inhibition results of antagonism, observing a bacteriostatic effect. The zones of inhibition were up to 17mm in length.

Conclusions: Bacteria isolated from marine organisms, water and sediment have antagonistic effect against *V. parahaemolyticus*, which is very important as a probiotic in shrimp farming to reduce the disease and the premature death of these crustaceans, this alternative may prevent millionaire losses of the production of shrimp farming.

Characterization of Merlin, a regulator of actin dynamics, in *Aedes* spp., mosquito vector of arboviruses

Febe Elena Cázares-Raga[1], Irving Ulises Martínez-Vargas[1], Antonio Celestino-Montes[1], Ángel Rubio Miranda[1], Abel Trujillo-Ocampo[1], Fernando Medina-Ramírez[1], María Eugenia Pérez-Bonilla[2], Rosa María del Ángel[1], Fidel de la Cruz Hernández-Hernández[1]. [1]Departamento de Infectómica y Patogénesis Molecular, Cinvestav-IPN, Av. IPN # 2508, Col. San Pedro Zacatenco, Ciudad de México 07360. [2]Escuela de Biología, Benemérita Universidad Autónoma de Puebla, Puebla 72570. fcazares@cinvestav.mx

The mosquitoes of the genus *Aedes* spp. are major vectors of several deadly arboviruses as Dengue (DV), chikungunya and Zika, affecting hundreds of millions of individuals annually in tropical and sub-tropical regions in addition, they are increasing their adaptation to urban areas, representing important public health problems. *Aedes* spp. requires for reproduction that mosquito females ingest blood and this behavior is determinant for pathogens transmission, because infectious agents invade mosquito when the host is infected, and they are transmitted when mosquito obtain blood from a new host. The most efficient strategies for the control of vector-borne diseases are directed against mosquito, such as the application of insecticides, but this method have profound disadvantages because select populations of resistant mosquitoes and causes environmental pollution; in addition, other control measures as vaccines and drugs are ineffective for these arboviruses caused diseases. Hematophagy and insecticides, among other xenobiotics, and viral infections are stress conditions that modify the expression of proteins by activating signaling pathways in insects. The ERM proteins (Ezrin-Radixin-Moesin) and Merlin (Moesin-Ezrin-Radixin-like protein) are components of the cellular cortex that connect plasma membrane proteins with the cytoskeleton, regulating diverse cellular functions, including stress responses and cell signaling. There is evidence that in mammalian cells ERM/M proteins regulate the entry and development of hepatitis C flavivirus. Previously, in the midgut of *Anopheles albimanus* mosquitoes we observed the diminished expression of ERM/M proteins and their association with actin during blood feeding (BF), event that causes mechanical stretch and probably induce this molecular behavior. In this work Merlin, also named neurofibromatosis type 2 (NF2) protein, was identified in mosquitoes *Ae. aegypti* and C6/36 HT cell line derived from *Ae. albopictus*. Using bioinformatics strategies, sequences of two genes and their hypothetical proteins annotated as Merlin/ERM were identified in the genome of *Ae. aegypti*; using a specific antibody to mammalian Merlin, an 80 kDa band (predicted molecular weight) was identified by WB/1D in all phases of the mosquito life cycle, and location of the protein was observed by immunofluorescence (IF) in several organs. In C6/36 HT cells, a similar band was detected by WB/1D and a spot with pI 5.0, by WB/2DE, more basic than predicted, possibly due to phosphorylation, was observed. It is known that cytoskeleton is modified and frequently co-opted by virus infection and ERM/Merlin proteins participate in the viral infection. These data suggest that Merlin could be activated in mosquito after infected BF and we are interested to test the hypothesis that this molecule plays a role during arbovirus infection of mosquito cells.

Characterization of bacteria associated with the rhizosphere of amaranth from a crop with a forest matrix in Tochimilsolco, Puebla

* Verónica Cepeda Cornejo [1], Isaac Michael Cantú Pérez[1], Isaac Hernández Sánchez [1], Kenneth Islas Ordoñez [1], Pablo Márquez Osorio [1], Miriam Mariel Sánchez García [1], Enrique Ortiz Torres [2], Lorena Milflores Flores [1,] Dalia Molina Romero [1], Berenice Venegas Meneses [1].
[1]Facultad de Ciencias Biológicas, Benemérita Universidad Autónoma de Puebla.
*veronicacepedac@gmail.com. Laboratorio 307 Cultivo de tejidos, Edificio Multilaboratorios 6 (EMA 6). Facultad de Ciencias Biológicas, Biotecnología, Benemérita Universidad Autónoma de Puebla. Blvd. Valsequillo y Av. San Claudio, Ciudad, Universitaria, Col. Jardines de San Manuel, C. P. 72570, Tel: 01 (222) 2295500 Ext. 3978.
[2]Colegio de Postgraduados, Campus Puebla.

Amaranth is one of the crops ancestral that is still cultivated in diverse localities of the central region of Mexico, including the states of Tlaxcala, Puebla, State of Mexico, and Mexico City, among other states. The seeds have a great nutritional value, they contain proteins, vitamins A, B, C and D; in addition to the essential amino acid Lysine that is rare in other cereals. Various foods are obtained from the seeds and include the traditional joys, flour for the preparation of breads and cookies, among others. The leaves are consumed as vegetables and are rich in iron, calcium, magnesium and vitamins A and C. Despite the importance of amaranth in food, little is known about the diversity and function of the microorganisms associated with the rhizosphere. The microbial diversity that grows in the soil and interacts with amaranth is important in order to know the diversity of growth promoting bacteria. The objective of this study is the characterization of a community of bacteria associated with the rhizosphere of amaranth as a previous study to search for activity of growth promoters. Methodology: The collection of soil associated with the rhizosphere of nine amaranth plants was carried out un plants growth in amaranth field in a forest environment, 3 kilometers away from the community of Tochimilsolco, Puebla. The isolation of microorganisms present in the soil was done by adding 1 gram of soil in 10 ml of 0.8% saline solution, three serial dilutions were made. Just 100 µl was inoculated on Luria Bertani solid medium and incubated for one to two days at 25 ° C. The colonies were characterized by morphology, color, consistency and Gram stain. The morphology was observed to the stereoscope and optical microscope. Results: A total of 47 colonies of different appearance were characterized from the soil samples. The results of the Gram stain showed that 60% of the microorganisms were Gram positive and the remaining 40% were Gram negative. The morphologies observed under the optical microscope (100x) indicated that the microorganisms were: Isolated Bacillus, Cocobacillus, Diplobacillus, Filamentous and Streptobacillus; being isolated bacilli (17%) the most recurrent. Conclusion: The reports in the literature indicate that among the microorganisms reported as growth promoters are bacilli and even some of the genus Serratia. These groups have been found in this study and we could expect that in future some experiments with these microorganisms present growth promoting activity.

The Abundance of bacteriophage *CrAssphage* in Mexican childrens with obesity it is related with enteric bacteria of gut microbiota

Melany J. Cervantes Echeverria, Shirley Bikel, Gamaliel Lopez-Leal, Samuel Canizales-Quinteros[2], Adrián Ochoa-Leyva [1]
[1]Departamento de Microbiología Molecular, Instituto de Biotecnología, UNAM. C.P. 62210. Cuernavaca, Morelos. México.
[2]Instituto Nacional de medicina Genómica.
[1]Corresponding author E-mail aochoa@ibt.unam.mx tel: +52 (777) 329-1668

The human intestine is a complex organ to achieve an adequate nutrient incorporation into the human diet. In the intestine there is a vast microbial ecosystem including species that permanently colonize it and many others acting as transient bacteria. Both of them bacterial populations provides different benefits to the host, such as optimizing the absorption of nutrients, the maintenance of the intestinal epithelium integrity, harvest energy of the diet, protection against pathogens, and the regulation of the immune system, among others.

Massive sequencing methods have led to characterize the bacterial composition of the intestinal microbiota and they found that presence and abundance of different bacterial taxa is modified under conditions of host disease, such as diabetes and obesity. In addition to bacteria, the human intestine is colonized by a wide variety of viruses, mainly of prokaryotic viruses named bacteriophages, which can use the intestinal bacteria for their replication.

Recently, a 97,065 bp genome of a bacteriophage called CrAssphage was discovered from unknown sequences of human intestinal metagenomes. CrAssphage has attracted attention due the genome is highly abundant comprising up to 90% and 22% of all reads in virus-like particle (VLP)-derived metagenomes and total metagenomes. This phage was reported from children aged <1 month to adults. Bioinformatic analysis predicted that its possible phage-host is members of Bacterioidetes phylum, but its role in diseases such as obesity with metabolic syndrome is unknown. In our research, we have performed bioinformatics and statistical analysis of viral DNA sequences obtained from 28 fecal samples of Mexican children with obesity, obesity plus metabolic syndrome and normal weight. We searched for significant differences in the abundance and presence of CrAssphage among three phenotypes. In addition, 16s rRNA gene sequences have been analyzed to know the bacteria in each sample. Correlations were made to determine if there is any relationship between the abundance of CrAssphage and the presence and absence of certain intestinal bacteria.

Analysis of variance between phenotypes indicated that CrAssphage abundance does not vary significantly among children with normal weight, obesity and metabolic syndrome. This provides a landscape about the interaction between the bacteriophage and the intestinal microbiota in Mexican children.

Comparative study between diarrhoegenic Escherichia coli virotypes of the México City with virotypes of San Luis Potosí

Ruth Adriana Cervantes-Olmos, Carmen Mercado Alquicira, Patricia Arzate-Barbosa, Ana María Castro.
Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México, México.
Jefe de grupo: Dra. Ana María Castro.
Datos: Av. Universidad 3000, Col. Universidad Nacional Autónoma de México, C.U. Delegación Coyoacán, CD.MX, C.P.04510.
Número cel: 0445514324652
Correo: amcastro@unam.mx

Abstract

The distribution and frequency of *Escherichia coli* virotypes can vary considerably from region to region, due to the geographical characteristics, the degree of social lag as well as the population of each region. Therefore, the objective of the present work was to compare *E. coli* isolated from children with diarrhea obtained from two states with different levels of social lag and establish virotypes, adhesion to HEp-2 cells, biofilm formation and antimicrobial resistance of this bacterium.

In the present work, we compared *E. coli* isolated from children with diarrhea obtained from the City of San Luis Potosí with *E. coli* isolated from children with diarrhea of CDMX, with the purpose of identifying and establishing virotypes, adherence to HEp-2 cells, formation of biofilms and antimicrobial resistance of these bacterias. The results showed that the percentage of virotypes found in S.L.P., were higher with respect to the virotypes found in the CDMX, being ETEC the most frequent virotype for both populations. The incidence of this virotype was higher for *E. coli* isolated from S.L.P., and the statistical analysis showed $p < 0.000$. The incidence of the EIEC and EHEC virotypes was higher in the state of S.L.P., with $p < 0.000$. The adhesion phenotype, both aggregative and diffuse, was related to the presence of the *aat* and *astA* genes. The tests to determine the production of biofilms in strains of *E. coli*, allowed us to determine that the method in Congo red agar, was the most effective for its evaluation. In relation to the antimicrobials tested, a higher percentage of resistance of the *E. coli* strains of the CDMX was observed, in relation to the strains of S.L.P., with a statistically significant difference of $p < 0.05$.

Molecular characterization, phylogenetic analysis and replication of a new begomovirus infecting bean in Nayarit, Mexico.

Martínez-Marrero Nadia[1], Avalos-Calleros Jesus Aaaron[1], Chiquito-Almanza Enrique[2], Acosta-Gallegos Jorge A.[3], Ambriz-Granados Salvador[1], Arguello-Astorga Gerardo Rafael[1], Anaya-López Jose Luis[3]*
[1]División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica, Camino a la Presa San José, 78216 San Luís Potosí, San Luís Potosí, México,
[2] Departamento de Ingeniería Biquioquímica, Instituto Tecnológico de Celaya, C.P. 38010, Celaya, Gto., México; [3]INIFAP-Campo Experimental Bajío, C.P. 38010, Celaya, Gto., México.
*Correspondencia: jose.luis.al@hotmail.com; nadia.martinezm@ipicyt.edu.mx

In january 2014 a sample of a bean plant (*Phaseolus vulgaris,* cv. Mayocoba) exhibiting symptoms of virosis was collected in an agricultural field of Nayarit, Mexico. The siRNAs of this sample, CN-30 (BioProject- NCBI accession PRJNA362733) were sequenced and assembled in contigs. Preliminary in silico analysis suggested the presence of several different begomoviruses. However, the subsequent analysis of sample CN30 by lineage-specific overlapping PCR (LISOP) revealed the presence of only one bipartite begomovirus. Full-length clones of the two genomic components (DNA-A and DNA-B) were obtained by RCA, and sequenced. Comparing the sequence of the complete DNA-A against the Genbank database, revealed that the closest relative of this begomovirus displays a sequence identity below 81%, hence indicating that it is a new begomoviral species. The DNA-A sequence contains the five expected open reading frames (AC1, AC2, AC3, AC4 and AV1) and an intriguing putative ORF overlapped in the AV1 gene (172-531 nt). In the DNA-B the two expected ORFs (BV1 and BC1) are present. Arrangement of *cis*-acting elements in promoters was also analyzed. The presence of siRNAs from at least two different RNA viruses in sample CN-30 were also detected; consequently, the observed symptoms were not caused only by the isolated begomovirus. In order to determine the actual symptoms in bean plants infected with that virus, the genomic components were cloned in a pBlueScript vector (DNA-A in the site *BamHI* and the DNA-B in the site *ClaI*;) and partial tandem dimeric constructs were generated for each component, designated VFDNA-A 1.84 and VFDNA-B 1.72. These infectious constructs were inoculated by agroinfiltration in *Nicotiana benthamiana* plants and the replicaction of the monomer was confirmed. In addition, the infectious constructs will be inoculated by agroinfiltration in plants of several cultivars of beans. The symptoms and their kinetics of appearance will be examined.

Key words: Bean virus, begomoviruses, new species.

Management of patients infected with HIV in the State of Tabasco.

Luis Manuel Contreras Cabrera[2], José Arnold González Garrido[2], Cesar Augusto Reyes Garcia[1], Carlos Javier López Victorio[2]. 1.Secretary of Health of the State of Tabasco. [2].Universidad Juárez Autónoma De Tabasco. Academic Division of Basic Sciences. Road Cunduacán-Jalpa KM. 1 Colony. The Emerald PC. 86690. Phone. 5539965215, javier.lopez@ujat.mx.

Viruses are constituted by a fragment of DNA or RNA, whose size varies between 50-200 nm, some have a protein coat called a capsid; Depending on whether they have a membranous wrap they are called wrapped or naked if they lack it. The human immunodeficiency virus (HIV) is a retrovirus lentivirus. People infected with HIV can develop at some point a severe immunodeficiency syndrome known as Acquired Immune Deficiency Syndrome (AIDS). This virus is transmitted via: sexual, perinatal and blood. HIV attacks the immune system of the infected person. Destroying the CD4 lymphocytes. The HIV virus binds to the cell membrane, fusing its capsid, introducing its genetic material to multiply it, which causes a reduction of CD4 cells and an increase in the number of copies of the virus circulating in the blood (viral load) producing an immune deficiency. In Mexico there are units of the Outpatient Center for Prevention and Care in AIDS and Sexually Transmitted Infections (CAPASITS) that provide prevention services, medical attention, psychological support in addition to providing free medicines. Antiretrovirals (AR) are reduce the risk of progression and infection of HIV. Usually 3 types of AR are used; inhibitors of nucleoside reverse transcriptase (NRTI), and non-nucleoside (NNRTI), and protease inhibitors (PI). It is estimated that there are 33.2 million people in the world infected with HIV, in the last 20 years 223,995 cases of people infected in Mexico have been reported, of which 116,936 were alive, 94,812 deceased and 12,247 are unknown of their current situation.[1] Currently in the state of Tabasco, 4,206 HIV cases are registered, of which 88% are male, the main transmission mechanism is via sexual activity with 93%, followed by 7% perinatally, 78.13% of infected patients are in a range between 15 and 44 years of age. Out of the total of 3922 registered patients, they received various basic AR treatment schemes consisting of an NRTI combination; LAMIVUDINE, LOPINAVIR / RITONAVIR, EMTRICITABINE / TENOFOVIR, EFAVIRENZ, ABACAVIR / LAMIVUDINE on the other hand the combination of NRTI, NNRTI; EMTRICITABIN / TENOFOVIR / EFAVIRENZ, is the main combination prescribed with 2160 doses being this the main scheme of attention prescribed.

1 SS/CENSIDA. (2014). Directorate of Operational Research, based on the Epidemiological Surveillance System for HIV and AIDS (SS/DGE/SUIVE).

Importance of host cholesterol in the DENV, ZIKV and YFV infection

LBM. Carlos Daniel Cordero-Rivera[1], M.C. Juan Fidel Osuna-Ramos[1], Biol. Carlos Noe Farfan-Morales[1], M.C. José Manuel Reyes-Ruiz[1], D.C. Rosa Maria del Ángel[1].
Departamento de Infectómica y Patogénesis Molecular[1], CINVESTAV-IPN1, México,CDMX Código Postal 07360;
Tel. (525) 5747-7000 Ext. 5647 ó 5648; rmangel@cinvestav.mx

Flaviviruses are positive-sense single-stranded RNA viruses, many members of this family represent a significant threat to human health. Infectious agent as dengue fever virus (DENV), yellow fever virus (YFV) and Zika virus (ZIKV) are transmitted to humans by mosquitoes Aedes, which circulates in tropical and subtropical regions worldwide. Due to a vaccine absence or an effective drug to defeat these flaviviruses infections, these viruses are currently a major health problem. Then, the development of efficient strategies for their control is crucial.

During the life cycle, viruses depend on the well-functioning of many cellular processes and of different cellular components that they sequester for replication. Among those components, one of the most important is the cholesterol. Cholesterol is involved in several steps of the virus life cycle of many of the enveloped and even of the nonenveloped viruses. Previous works of our laboratory have reported that cholesterol is important for the establishment of DENV infection, and that DENV increase cellular cholesterol to promote its replication. Nonetheless, the role of cholesterol during ZIKV and YFV infection is yet unknown.

Therefore, the aim of this study was to assess and compare the capacity of DENV, ZIKV and YFV to modify cellular cholesterol levels during the infection in different cell lines. This was analyzed by flow cytometry, a colorimetric essay and confocal and electron transmission microscopy techniques. Additionally, the effect caused by different drugs already approved by the FDA, capable of inhibiting the synthesis and uptake of cholesterol, was also assessed during the infection of these three flaviviruses.

Our results indicate that the infection with DENV, ZIKV and YFV modify cellular cholesterol levels and that the use of FDA approved cholesterol-lowering drugs or cholesterol uptake inhibitors hampered viral infection, by affecting the formation of replicative complexes. In summary our results suggest that cholesterol is important for the replicative cycle of the flaviviruses DENV,ZIKV and YFV.

Expression of miR-142-3p and infection by *Helicobacter pylori* in patients with chronic gastritis and gastric cancer

Ana B. Cruz Hernández, María A. Camacho Miranda, Josefina Atrisco Morales, Dinorah N. Martínez Carrillo, Hilda Jiménez Wences, Adolfo Román Román, Gloria Fernández Tilapa.
Laboratorio de Investigación Clínica, Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Guerrero.
Av. Lázaro Cárdenas S/N, C.P. 39090, teléfono, 4725503, gferti@hotmail.com

Introduction. *Helicobacter pylori* induces an intense inflammatory response in the human gastric mucosa and evades the adaptive immune response. The anti-*H. pylori* response is regulated by microRNAs (miRNAs) and cytokines expressed by epithelial cells or the immune system. As posttranscriptional regulators, miRNAs inhibit the transcription of the white mRNA. miR-142-3p hybridizes with mRNA of PKCα and N-Wasp and thus interferes with phagocytes; on the other hand, it negatively regulates the activation factor of B lymphocytes, decreasing the production of anti-*H. pylori* antibodies.

Objective. To assess the expression level of miR-142-3p in patients with chronic gastritis and gastric cancer with *H. pylori* infection.

Material and methods. 31 patients were studied, 22 diagnosed with chronic gastritis and 9 with gastric cancer. Two gastric biopsies were obtained from each patient, one for diagnosis of *H. pylori* infection, its *vacA* genotypes and the *cagA* status by PCR and the second to determine the expression of miR-142-3p by RT-qPCR**.**

Results. The overall frequency of *H. pylori* was 48.3% (14/31), 40.9% (9/22) in chronic gastritis and 55.5% (5/9) in gastric cancer. 78.6% (11/14) of those infected had genotype *vacA s1m1/cagA*+ genotype**,** and this one was found in 77.7% (7/9) and 80% (4/5) of *H. pylori*+ with gastritis and cancer, respectively. In patients with chronic gastritis relative expression of miR-142-3p was significantly higher (p = 0.0001) than in gastric cancer. Don´t differences were found in expression level of miR-142-3p in chronic gastritis (p = 0.1153) and cancerous tissue (p = 0.9048) negative and positive to *H. pylori*.

Conclusions. The expression of miR-142-3p is higher in patients with chronic gastritis than with gastric cancer and it´s not related to *H. pylori* infection. The expression of miR-142-3p is higher in inflammatory states.

Key words: *Helicobacter pylori*, miRNAs, immune response, miR-142-3p.

Comparative analysis of biofilm production by *Sporothrix schenckii* conidia and yeast-like cells.

Marilyn Gwendolyne De los Santos-Rojas, Héctor M. Mora-Montes, Gustavo Alexis Niño-Vega. Departamento de Biología, División de Ciencias Naturales y Exactas, Universidad de Guanajuato, Noria Alta s/n, Col. Noria Alta, Guanajuato, Gto, México. C.P. 36050. *Tel:(473) 73 20006 ext. 1452, e-mail: gustavo.nino@ugto.mx

Biofilms are described as highly structured communities of microorganisms that are associated with a surface linked together through an extracellular matrix. Its formation allows the microorganism protection against environmental, chemical or physical stress, metabolic cooperation and regulation of the expression of genes, such as those involved in adhesion to surfaces, a key step in the formation of biofilms. In recent years, there has been a significant increment in the reports of diseases caused by both filamentous and yeast fungi capable of forming biofilms. Among them are species of *Candida, Aspergillus, Cryptococcus, Pneumocystis, Coccidiodes,* and *Paracoccidiodes*. This type of structure differs according to the species. It allows the fungus a high tolerance to drugs and ability to adhere to surfaces of living matter (such as endothelium) and non-living, such as medical devices (catheters) making biofilms a relevant subject of study.

Sporotrichosis, a mycosis caused by dimorphic fungi of the genus *Sporothrix* that affects humans and animals, has a cosmopolitan distribution, although it occurs mostly in tropical and subtropical regions, and is one of the most frequent endemic fungal diseases in America. It can be transmitted by trauma with contaminated vegetative material or by bites or scratches from infected animals. *Sporothrix spp.* has been found in its parasitic phase in catheters of hospitalized people. In addition, there are reports of yeast transmission of *Sporothrix spp.* from cats to people.

From the *Sporothrix* complex, *S. schenckii* has been reported as a strong producer of *in vitro* biofilms from conidia, showing resistance to the antifungals commonly used for treatment. In addition, *S. schenckii* has shown an ability to form biofilm-like structures from yeast (unpublished data). Taking into account that most of the information on fungal biofilms has been generated in *in vitro* models, and that there are reports in which they present a structural similarity with biofilms produced *in vivo*, the comparison of biofilms formed from yeasts and conidia of *S. schenckii in vitro*, could be a first step to understand a possible role of this type of fungal growth in the establishment and development of the infection.

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Vaccine efficacy of BCG-Phipps in bovine tuberculosis by monitoring response to ESAT-6 and CFP-10 antigens in IFN-γ release assay

Fernando Díaz Otero[1]*, Laura Jaramillo Meza[1], Alberto U. Espinosa Alaníz[2], Rafael Pérez González[2]
[1]CENID-Microbiología Animal INIFAP. Carretera Federal México-Toluca Km. 15.5, Col. Palo Alto, Delegación Cuajimalpa, Cd. de México, CP 05110. Tel. 5255 38718700 Ext. 80315; 2FES-Cuautitlán-UNAM.
E mail: diof0009@yahoo.com.mx

Bovine tuberculosis (BTb) has a direct impact in the productive and reproductive efficiency of dairy cattle, causing major economic losses in agriculture. Nowadays, the control of the disease is based on a test and slaughter strategy, an unaffordable method for undeveloped countries, since economic resources are well below the requirements for removal of the positive cattle herds. Because of this, immunization with the BCG vaccine represent the best control alternative. However, there is the disadvantage that its use can induce reactivity to the tuberculin test in the bovine population to which it is applied. In this regard, ESAT-6 and CFP-10 antigens encoded by the RD1 region present in the species of the *M. tuberculosis* complex but absent in substrains of BCG are considered candidate antigens for differential diagnosis of vaccinated and infected cattle. The objective of the study was to monitor the protection induced by the BCG vaccine by evaluating the response to ESAT-6 and CFP-10 antigens from vaccinated calves in blood-based interferon-gamma (IFN-γ) release assays (IGRAs).Thirty-five calves were vaccinated with BCG-Phipps (10^6 CFU/1.5 ml) and had thirty unvaccinated calves, as a control group. Both groups were monitored for seven months to evaluate IFN-γ production in whole blood cultures stimulated with antigens referred. The IFN-γ released in the cultures supernatants was quantified by sandwich ELISA. T-Student was applied for the comparison of results in the different sampling times. There were no differences in levels of IFN-γ to antigens between groups during the evaluated period. However, an increase towards ESAT-6 was observed in one of the animals vaccinated at day 30 pv. In unvaccinated animals, a high and sustained production was observed for ESAT-6 in two calves, in one from the beginning of the study until day 30, and in the other, from day 7 to day 21 with oscillating values. The levels did not rise again during the rest of the study for none of the groups. The low and intermittent responses to ESAT-6 and CFP-10 may be due to the sensitization by non-tuberculous mycobacteria, several of which also contain the genes that encode these proteins and that could be circulating in the herd, since no vaccinated or infected animal was isolated *M. bovis*. According to the results, BCG vaccine had a protective effect during the assessment period, so its use for disease control would be suitable. There is the disadvantage that its use can induce reactivity to the tuberculin test in the bovine population to which it is applied. In relation to this it has been indicated that the referred antigens are suitable candidates for the differential diagnosis among cattle vaccinated from infected. Mostly, it has been shown that these antigens are important targets of T cells and strongly induces the production of IFN-γ in tuberculous cattle, so its use in diagnostic tests of IFN-γ release improves its sensitivity.

Evaluation of VP4 expression in MA-104 cell line infected with rotavirus.

Tania Guadalupe Heredia Torres, Carlos Eduardo Hernández Luna, María Cristina Rodríguez Padilla, Reyes Silvestre Tamez Guerra y Juan Francisco Contreras Cordero.
Universidad Autónoma de Nuevo León. Facultad de Ciencias Biológicas. Pedro de alba S/N, Ciudad Universitaria, San Nicolás de los Garza, Nuevo León. CP 66455. +528183294000 Ext.6432.
Email: tania.ght@hotmai.com

Introduction: During infection with rotavirus, the affected cells go through cycle arrest and the cellular machinery is used by the virus for its replication, production of viral proteins and formation of new viral particles. VP4 is a viral protein on the surface of the virion and it mediates the attachment and penetration of rotavirus on the cells. The production of this protein is essential for the spread of the virus.

Objective: Evaluate the expression of VP4 produced by rotavirus infection in the MA104 cell line. **Materials and methods:** MA-104 cells were infected with rotavirus Wa strain with an MOI of 0.1, aliquots were taken at different times of infection, RNA was extracted for a polyacrylamide gel electrophoresis. The presence of viral proteins was carried out by SDS-PAGE and for the detection of VP4 a Western Blot was performed.

Results: The MA-104 infected cells showed a marked cytopathic effect from 24 to 48 hours post infection. The major viral production reflected in the electrophoresis was at 48 hours post infection. However, the SDS-PAGE indicated decrease in cellular proteins and increase in viral proteins, including VP4, from the first 6 hours of infection. Western Blot reflected the production of VP4 during viral infection at 6 hours post infection. **Conclusions:** Rotavirus produces VP4 from the first hours of infection allowing the formation of new viral particles and the spread of the virus.

Participation of the *pvd* gene cluster in the mechanism of Quorum sensing and virulence of *Pseudomonas aeruginosa* PAO1

Sharel Pamela Díaz Pérez, Claudia Adilene López Hernández, Cristhian Said Solís Ortíz, Alma L. Díaz Pérez, Jesús Campos García.
Laboratorio de Biotecnología Microbiana, Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Ciudad Universitaria, 58038, Morelia, Mich., México. Phone/Fax: +52 443326 5788, ext. 120. E-mail: jcgarcia@umich.mx

Abstract. *Pseudomonas aeruginosa* is an opportunistic pathogenic bacterium for humans, animals and plants. This bacterium possesses a system of perception of bacterial quorum (quorum sensing, QS). The LasI/LasR QS system regulates the production of the cyclodipeptides (CDPs) cyclo(L-Pro-L-Val), cyclo(L-Pro-L-Phe) and cyclo(L-Pro-L-Tyr). Although CDPs are molecules with bioactive properties, there are few elements of concern in the mechanism of bacterial biosynthesis and the roles that play in communication with host cells. In addition, it has been described that the CDPs production in *P. aeruginosa* mainly depends of the multi-modular non-ribosomal peptide synthetases (MM-NRPS), whose products such as the aeruginaldehyde have been involved in the bacterial QS mechanisms. In this study, was found that levels of pyocyanin (a virulence factor in *P. aeruginosa*) was increased in the *pvdJΔ* mutant strain, which encodes for a NPRS. The *pvd* gene cluster is directly related to the pioverdin synthesis, which is a fluorescent peptide-siderophore involved in the iron acquisition, being essential in the bacterial pathogenicity on the host. Therefore, is important to elucidate the mechanism by which the CDPs of *P. aeruginosa* PAO1 contribute to its pathogenesis and/or virulence. Production of different virulence factors of *P. aeruginosa* PAO1 with different single and double mutants in *pvdI, pvdJ* and *pvdL* genes were compared. The *pvdIΔ, pvdLΔ* and *pvdLΔ/pvdIΔ* mutants decreased 50% their production of biofilm (virulence factor) with respect to the PAO1 wild type (WT) strain. Likewise, the production of rhamnolipids was significantly increased in the simple mutant strains *pvdJΔ* and *pvdLΔ* compared with the WT strain; while that the mutant *pvdJΔ/pvdLΔ* showed similar rhamnolipids levels with the WT strain. Survival assays in *Caenorhabditis elegans* showed that the *pvdJΔ* mutant supernatants increases the death of the nematode in 40% compared with WT strain. In conclusion, the *pvd* gene cluster from *P. aeruginosa* is involved in the production of CDPs, suggesting that these regulate the production of virulence factors pioverdin, biofilm and rhamnolipids.

Keywords: **virulence, cyclodipeptides, pioverdin, quorum sensing.**

Molecular characterization of the rOrf1 protein of enteropathogenic *Escherichia coli*

Norma Espinosa Sánchez, Mariana Ríos Vázquez, Arely Ivonne Marcos Vilchis, María del Pilar Torres Reyes and Bertha González Pedrajo
Instituto de Fisiología Celular, Universidad Nacional Autónoma de México. Circuito Exterior s/n, C.U., Coyoacán C.P. 04510, Mexico City.
bpedrajo@ifc.unam.mx

The human gut microbiome is composed of different types of bacteria, some of them having a symbiotic relationship with the host, such as *Escherichia coli*. However, some bacteria have acquired virulence factors and to date, eight *E. coli* pathovars have been described, one of them is enteropathogenic *E. coli (*EPEC). This bacterial pathogen belongs to the attaching and effacing (A/E) family and is one of the principal causative agents of diarrhea in developing countries, where infants from 0 to 11 months are the most affected.

EPEC virulence relies on a molecular nanomachine known as the type three secretion system (T3SS). The T3SS enables bacteria to inject proteins (called effectors) directly into the cytoplasm of enterocytes in order to control cellular processes for its own benefit.

The biogenesis of the T3SS is highly regulated and the structural components, as well as transcriptional regulators, effectors, chaperones and proteins that participate in secretion regulation are encoded by a 35.6 kb pathogenicity island called LEE (Locus of Enterocyte Effacement). The LEE contains 41 genes organized in 7 operons (*LEE1* to *LEE7*) and four monocistronic units.

All the LEE encoded proteins have been characterized except for one of 31 kDa called rOrf1. Therefore, the aim of this work is to study the role of the rOrf1 protein in the T3SS. The *rorf1* gene is located in the *LEE6* bicistronic operon, next to the *espG* gene encoding a type III effector. We generated an *rorf1* null mutant which presented the same secretion pattern as the wild-type strain. Here we will present the functional characterization of the rOrf1 protein.

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Silencing SOCS1 and SOCS3 in T Helper cells resulted in augmented Interleukin 10 production during Dengue virus infection

Alicia Marquez-Bandala[1,2], Julio Reyes-Leyva [2], and Lilian Flores-Mendoza [2, 3]*.
[1] Centro de Investigación en Ciencias Microbiologicas - Benemérita Universidad Autónoma de Puebla.
[2] Centro de Investigación Biomédica de Oriente CIBIOR-IMSS.
[3] Departamento de Ciencias Químico Biológicas, Universidad de Sonora, Unidad Regional Sur.

Keywords: Dengue, SOCS proteins, T cells.

Introduction: It has been proposed that T cells play an important role in the immunopathogenesis of dengue disease. A predominant Th1 response is thought to occur during the initial stage of infection while a Th2 response tends to appear later. The response to cytokines is regulated by proteins known as Suppressors of Cytokine Signaling SOCS. SOCS1 and SOCS3 proteins have been linked to the imbalance of cytokine response in acute viral infections, however their role in DENV infection has not been studied.

Objectives: To silence socs1 and socs3 genes in T lymphocytes and evaluate silencing effect during an in vitro DENV infection.

Methods: PBMC obtention was carried out using Ficoll gradient separation technique.

Monocyte derived dendritic cells were differentiated using Interleukin4 (IL4) and Granulocyte-Monocyte Stimulating Growth Factor (GMCSF). Dendritic cell immunophenotyping was carried out using monoclonal antibodies. For socs1 and socs3 silencing, concentrations of 2.5nM, 5nM y 10 nM of siRNA were evaluated. Co culture assays between infected dendritic cells were performed with dengue serotype 2 virus (MOI 1) and silenced T lymphocytes for 96 hours. For IL10 quantification, culture supernatants were collected every 12 hours. Additionally, proliferation assays were performed.

Results: Optimal concentration for inducing cell differentiation corresponds to 50 ng/mL GM-CSF and 45 ng/mL IL4. Differentiated cells phenotype was CD80+, CD86+, CD83+, CD32+, HLADR+ and CTLA4+. Optimum concentrations of siRNA correspond to 2.5 nM and 5nM for socs1 and socs3 respectively. Increased levels of IL10 were detected in cell cultures were SOCS1 and SOCS3 were silenced but these were lower than levels observed in non-silenced cells cultures, also, increased proliferation of lymphocytes was observed in silenced cells.

Conclusions: socs1 and socs3 silencing induces an increased IL10 production in dendritic cell and T lymphocytes co cultures. Overexpression in CTLA4+ in dendritic cells may indicate the induction of T cells anergy.

First report of the *Aedes aegypti Flavivirus* in field population of *Aedes aegypti* in Mexico, a mosquito-specific virus.

Suemy Flores-Ruiz[1]; Nohemi Cigarroa-Toledo[1]; Carlos Baak-Baak[1]; Angélica del R. Pech-May[2]; Daniel Nuñez Avellaneda[1]; Julián García-Rejón[1], Carlos Machain Williams[1]*.
[1]Laboratorio de Arbovirología, Centro de Investigaciones Regionales –Dr. Hideyo Noguchi" Inalámbrica. C.P. 97069. Tel. 9-24-59-10 ext.1260
*Corresponding author: carlos.machain@correo.uady.mx
[2]Instituto Nacional de medicina Tropical, Neuquén y Jujuy s/n. Puerto Iguazú. Misiones-Argentina

Introduction. Mosquito-specific virus are viruses that naturally infect mosquitoes and replicate in their cells, but do not appear to replicate in vertebrate cells or infect humans or other vertebrates. Experimental studies with the insect-specific flavivirus like *Culex flavivirus* suggest that could potentially modulate arbovirus transmission and could play an important role in regulating the mosquito abundance or vector competence.

Objective. Identification by Reverse transcription polymerase chain reaction (RT-PCR) the NS5 region of Flaviviruses in field population of *Aedes aegypti* from Yucatan, Mexico.

Methodology. Adult mosquitoes were collected indoor and outdoor of the houses and were identified using taxonomic keys. Females *Ae. aegypti* (579) were pooled (89) for RNA extraction. Total RNA was analyzed by using reverse transcription PCR (RT-PCR) and using flavivirus-specific primers which amplify an ~251 nt region of the NS5 gene. RT-PCR products were purified, and Sanger sequencing was performed. Forward and reverse sequences from the samples were used to generate consensus sequences. These sequences were manually aligned and edited using Mega v.7. Then were compared to other sequences in the Genbank database using the Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The analysis of phylogenetic inference was made in the program Mr. Bayes v.3.2

Results. Analysis of alignment showed that three pools had 98.2 – 100% similarity and 98.8 –100% identity of nucleotide swith the sequences of *Aedes aegypti* Flavivirus (AeFv) reported in Portugal (Genbank Accession Nos. HQ676624 and HQ 676625). The phylogenetic analyzes showed that two sequences of the AeFv are the same variant for Yucatan. This is the second report of AeFV in Aedes aegypti mosquitoes and the first report of this virus in Yucatán, Mexico.

Conclusions. In worldwide, it is second report of AeFv in *Ae. aegypti* and first report in field population of *Ae. aegypti* in Yucatan, Mexico. AeFv is a poorly virus characterized and mosquito-specific that is grouped with the Flaviviridae family. It is suggested the isolation of this virus and determine vectorial competence.

Antiviral effect of a Dializable Leukocytes Extract in MDCK cells infected with influenza AH1N1 pandemic 2009

García Martínez Daniel [1], Pérez-Soto Elvia[1], Cabello Gutiérrez Carlos[2]; Gómez-García María del Consuelo[1], Pérez de la Mora Carlos Adolfo[3] y **David G. Pérez Ishiwara**[1*]

[1] Laboratorio de Biomedicina Molecular I, Programa Institucional de Biomedicina Molecular, ENMyH-Instituto Politécnico Nacional. Guillermo Massieu Helguera No. 239, Fracc, La Escalera, Col Ticomán, México D.F. C.P.07320. Tel. 01555729-6300 Ext.55534.
[2] Departamento de Investigación en Virología y Micología, INER.
[3] Farmainmune.
*Corresponding autor : ishiwaramx@yahoo.com.mx

Influenza causes elevated levels of morbidity and mortality worldwide due virus mutability. Disease outcome depends on the viral factors and the host immune response. Treatment is based on antiviral, vaccines and the use of immunomodulators. The Dialyzable Leukocyte Extract (DLE_{AA}) is an immune modulator constituted by a mixture of small peptides with anti-inflammatory, immune regulatory and putative antiviral effects. Here, we investigate the antiviral effect of DLE_{AA} against influenza AH1N1 pandemic 2009 (AH1N1 pdm09). Cytotoxicity of DLE_{AA} on MDCK cells was evaluated by MTT assay. Antiviral effect was done by preventive and treatment schemes using a MDCK AH1N1 infection model, performing hemagglutination assay and cytopathic effect at 24 and 48 hours post-infection (hpi). Results were analyzed by Graph Pad Prism software. We observed that non-infected cells formed a confluent monolayer with adherent morphology, however infected cells had cytopathic effect at 24 hpi and approximately almost all the infected cells appeared smaller and irregularly shaped at 48 hpi. In both, preventive and treatment schemes, DLE_{AA} protected cells against viral cytopathic effects. The quantification of viral particles showed that 0.001, 10 and 50 µg/mL of DLE_{AA} used as preventive scheme statistically diminished 54.86 ±6.39% the infectious viral particles 24 hpi and 64.12 ± 7.13 % 48 hpi in all evaluated concentrations. Also the use of 0.00025, 1 and 50 µg/mL of DLE_{AA} as treatment scheme significantly diminished viral titer 50% 24 hpi and 56.94±8.28% in all concentrations at 48 hpi. Together, the data presented here suggest that DLE_{AA} have an anti-influenza activity *in* vitro particularly when is used preventively using 0.00025 µg/mL. Thus, we proposed it as immunomodulator for prevention and treatment of influenza A.

Importance of co-infection of influenza virus-bactrias with host damage

Alexis Eduardo García García, Carlos Cabello Gutiérrez, Armando Pérez Torres, Ana María Castro.
Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México, México.
Jefe de grupo: Dra. Ana María Castro.
Datos: Av. Universidad 3000, Col. Universidad Nacional Autónoma de México, C.U. Delegación Coyoacán, CD.MX, C.P.04510.
Número cel: 0445514324652
Correo: amcastro@unam.mx

Abstract Acute respiratory infections (ARI) are one of the leading causes of morbidity and mortality in children under five years of age worldwide. Frequently, the viral infection is complicated by a bacterial infection and this co-infection causes severe pneumonia. Within these infections, the main etiological agents are viruses and bacteria. One of the viruses that most frequently causes ARI is the influenza virus, and within the bacteria *Haemophilus influenzae* and *Streptococcus pneumoniae*. If it is not treated in a timely manner, the disease evolves until the patient death. Given that the damage produced by the influenza virus is reduced to inflammation and necrosis of the respiratory tract epithelium, the purpose of the present work was to demonstrate that the co-infection of the influenza virus with bacteria that affect the respiratory tract is responsible for the multi-systemic damage. For this, six groups of mice were formed: one control group, one infected with the influenza virus, another two infected with bacteria with *H. influenzae* and *S. pneumoniae* respectively and two more co-infected with the influenza virus and bacteria, viruses-*H.influenzae* and virus-*S.pneumoniae* respectively. The study was followed for four weeks, sacrificing three mice from each group every three days. From the second day post-coinfection, the mice presented effects of the infections as hepatomegaly, splenomegaly abdominal hemorrhage, nodules in the lung, blood fluid in the pleura, blood in the intestine, etc. The fact that only the co-infection group with *S.pneumoniae* has presented damage, it is probably due to the virulence factors of this bacterium.

Similar mutations in LiaR are present in lipopeptide susceptible and resistant *Enterococcus faecium* isolates

María Carlota García-Gutiérrez[1]*, Francisco Benavides-Correa[1,2], Erika Netro-Baez[1,2], Susana Flores-Robles[2], David García-Gutiérrez[2], Juan Carlos Solís-Sáinz[1], Pablo García-S[1], Guadalupe Zaldívar-Lelo de Larrea de Larrea[1], Carlos Sosa Ferreyra[1]
[1]Departamento de Investigación Biomédica, Facultad de Medicina, Universidad Autónoma de Querétaro, México. C.P. 76177.
[2]Facultad de Química, Universidad Autónoma de Queretaro, Queretaro, México. C.P. 76010.
*mariacgg@yahoo.com

Introduction: *Enterococcus faecium* is one of the main nosocomial bacterias, grouped in the ESKAPE acronym, that cause Health-care Associated Infections. Due to its multidrug resistance, therapeutic options are limited, and relatively new antibacterial agents are been used to treat this type of infections. That is the case of Lipopeptides (Daptomicyn), which are used in cases of difficult to treat Gram positive infections, however, emergence of resistance to these agent had already been reported.

Objetive: To characterize, by microbiological and molecular methods, the resistance to lipopeptides in *Enterococcus faecium* nosocomial isolates.

Methods: Nosocomial isolates of *Enterococcus faecium* resistant to vancomycin obtained in two different years from the same public hospital at Queretaro city, were selected based on their resistance to daptomycin, including susceptible, borderline susceptible (MICs 3 and 4 µg/mL) and resistant isolates. Daptomycin susceptibility was compared by E-test in Mueller Hinton (MHA) and Brain Heart Infusion Agar (BHIA). Mutations in the liaFSR system genes were search by molecular biology and bioinformatics techniques.

Results: Daptomycin MICs in BHIA were 3 to 6 folds higher compared to those obtained in MHA in 96% of the isolates. Both, resistant isolates and those with borderline susceptibility, had some of the following changes of the coding sequences of LiaR gene: Trp73Cys, Ala45Thr, Lys75Glu and Asp142Glu. No translational changes were found in LiaF and LiaS sequences.

Conclusions: Here we show that the same translational changes in LiaR gen are present in both daptomycin borderline susceptible and resistant isolates. This finding suggests that the cut-off points and/or methods to determine lipopeptide suceptibility should be re-evaluated. Moreover, clinicians should be aware that borderline susceptible isolates could develop resistance during treatment due to the potential presence of these mutations.

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Biochemical and phylogenetic study of a flagellar lytic transglycosylase SltF from *Rhodobacter sphaeroides*.

Mariela García-Ramos[1]*, Javier de la Mora[1], Laura Camarena[2] and Georges Dreyfus[1].
Instituto de Fisiología Celular[1]. Instituto de Investigaciones Biomédicas[2].
Universidad Nacional Autónoma de Mexico. Circuito Exterior s/n, Coyoacan 04510 Mexico City, Mexico. 56225618 gdreyfus@ifc.unam.mx

Bacterial motility is mediated by rotating flagella that allow the cell to explore the medium searching for nutrients or to escape from harsh conditions.

Flagellar biogenesis is an orchestrated event where the structure spans the bacterial cell envelope. The diameter of the rod is approximately 4 nm which is larger than the estimated pore size of the peptidoglycan layer that must be penetrated. Therefore it requires the localized and controlled lysis of the cell wall. We found that a 47-residue domain of the C terminus of the lytic transglycosylase (LT) SltF of *R. sphaeroides* is involved in the recognition of the rod chaperone FlgJ. We also found that in many α-proteobacteria the flagellar cluster includes a homolog of SltF and FlgJ, indicating that the association of a LT with the flagellar machinery is ancestral.

In this work we show that SltF from *Rhodobacter sphaeroides* interacts with the scaffolding protein FlgJ in the periplasm through the C-terminal region. The characterization of the genetic context of *flgJ* and *sltF* in α-proteobacteria shows that these two separate genes show a tendency to coexist in a flagellar gene cluster. Two domains of unknown function in SltF were studied and results show that deletion of a 17 amino acid segment near the N-terminus does not show a recognizable phenotype whereas the deletion of 47 and 95 amino acid of the C-terminus of SltF disrupts the interaction with FlgJ without affecting transglycosylase catalytic activity of SltF. These mutant proteins are unable to support swimming indicating that physical interaction between SltF and FlgJ is central for flagellar formation. In a maximum likelihood tree of representative lytic transglycosylases all of the flagellar SltF proteins cluster in subfamily 1F. From this analysis it was also revealed that the lytic transglycosylases related to the type III secretion systems present in the pathogenesis gene cluster group with the closely related flagellar transglycosylases.

*This work was supported by CONACyT (CB-235996) and DGAPA (IN-204317)

Role of PqsE in the production of pyocyanin and elastase in *Pseudomonas aeruginosa* strain ATCC 9027, which belongs to the outlier PA7 clade

Selene García-Reyes[1], Alejandra A. González Valdez[1], Estefania Morales-Ruíz[1], Miguel Cocotl-Yañez[1], Gloria Soberón-Chávez[1]
[1]Instituto de investigaciones Biomédicas, Universidad Nacional Autónoma de México. Av. Universidad N°3000, C.P 04510 Ciudad de México, México. Corresponding: gloria@biomedicas.unam.mx

Pseudomonas aeruginosa is an opportunistic pathogen. Its pathogenicity is dependent on the production of several virulence factors (VF), such as elastase and pyocyanin that are dependent on the quorum-sensing (QS) response.

In the PAO1 type strain, the functioning of three QS systems (*las, rhl* and *pqs* systems) has been described. In this strain, the PqsE protein, that plays a key role in the synthesis of pyocyanin through the *rhl* system, is encoded within the *pqsA-E* operon. The expression of this operon is dependent on the transcriptional regulator PqsR. The aim of this work is to determine the role of the PqsE protein in the production of the VF elastase and pyocyanin in the *P. aeruginosa* ATCC 9027 strain, which belongs to the genetically diverse PA7 clade and is a natural *pqsR* mutant unable to produce both VF in Luria Bertani medium (LB).

Our hypothesis is that the mutation of *pqsR* prevents the expression of *pqsE*, and as a consequence the lack of PqsE does not permit the synthesis of pyocyanin and elastase by the *rhl* system. In order to determine if the lack of *pqsR* is responsible for the lack of pyocyanin and elastase production, ATCC 9027 strain was complemented with *pqsR*, and the production of these VF was measured. As expected, the synthesis of both VF was restored. In addition, when *pqsE* was expressed in this strain the results showed that PqsE protein was able to restore both VF. However, we found that in the PAO1 strain the presence or absence of PqsE does not affect elastase production. In the PAO1 reference strain the synthesis of elastase depends on the transcriptional regulator LasR, and our results obtained show that PqsE protein has a role in elastase synthesis in the ATCC 9027 contrary to what happens in *P. aeruginosa* PAO1. To determine if in strain ATCC 9027 the synthesis of elastase is also dependent on the transcriptional regulator LasR this protein was overexpressed and elastase was quantified. Surprisingly, the overexpression of the LasR did not contribute to the elastase synthesis in strain ATCC 9027. Our results enabled us to conclude that in *P. aeruginosa* ATCC 9027 the PqsE protein contributes to the regulation of the synthesis of pyocyanin and elastase. It was also shown that in this strain the production of elastase is independent of the presence of the transcriptional regulator LasR.

Thus it seems that elastase regulations is different in strain ATCC 9027 from the regulation previously reported for PAO1 strain depending on PqsE and not in LasR, but the pqsE-dependent regulation of pyocyanin is the same in both strains.

Identification and screening of *Trichoderma* strains natives from Querétaro with fungicide activity for biocontrol of phytopathogenic fungi.

Víctor Javier García Sánchez, Fidel Landeros Jaime, José Antonio Cervantes Chávez, Edgardo Ulises Esquivel Naranjo. Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro. Carretera a Chichimequillas s/n, Ejido Bolaños, Querétaro, Qro. C.P.76140. E-mail: ulises.esquivel@uaq.mx

The use of the biotic resources of an agroecosystem it has been stated as the best way to implement biocontrol of pests. Studies carried out with native strains of certain sites show a better effectiveness for the control of phytopathogenic fungi than using commercial strains. Genus *Trichoderma* is widely distributed, so the isolation and study of antagonism mechanisms of native strains constitutes an alternative to the use of inorganic fungicides. In addition, the application of metabolites instead of cells widens the area where it is planned to implement biological control and evades the harmful effects of *Trichoderma* over the mycobiota. The objective of this work is to identify and characterize strains of *Trichoderma* native to the state of Querétaro, as well as to evaluate the fungicidal effect of their filtrates on the phytopathogens *Rhizoctonia solani*, *Colletotrichum gloeosporioides*, *Sclerotium rolfsii* and *Fusarium oxsporum*. In total, 39 soil samples were collected sampled throughout Queretaro state, from which 133 strains of Trichoderma were obtained. Antibiosis assays were performed with the 133 strains to select and characterize the most effective strains. From the most effective strains, a mixture of their lyophilized filtrate was prepared to evaluate its fungicidal effect. At least 10 species belonging to the genus Trichoderma were identified through the use of concatenated trees. Two isolates corresponding to *Trichoderma asperellum* and *Trichoderma koningiopsis* showed an effect on the growth of phytopathogens greater than 75%. It was also determined that the highest antibiotic capacity of these strains is achieved in a rich medium (PDA), presumably due to the greater amount and diversity of carbohydrates in the medium. The filtrates of the strains retarded the development of mycelia of the phytopathogens and the germination of spores from a concentration of 300 µg/ml. These results suggest the presence of *Trichoderma* strains with potential application for the biological control of agricultural diseases through the use of their filtrates as biofungicides.

Analysis of the co-participation between the Epstein Barr virus and the bacterium *Helicobacter pylori* in a gastric cancer model

Ana Karen Gaytán-Jiménez*[1,3], Itzel Selene Herrera-Morales*[1,4], María Isabel Tussié-Luna[1,2,3] and Ezequiel M. Fuentes-Pananá[1,3].
[1]Unidad de Investigación en Virología y Cáncer, Hospital Infantil de México Federico Gómez, Dr. Márquez 162, Col. Doctores, 06720 Cuauhtémoc, Ciudad de México, México
[2]División de Investigación, Facultad de Medicina, Universidad Nacional Autónoma de México, Av. Universidad #3000. Col. Universidad Nacional Autónoma de México, C.U. 04510, Coyoacán, Ciudad de México, México.
[3]Programa de Maestría en Ciencias Bioquímicas, Universidad Nacional Autónoma de México, Ciudad de México, México.
[4]Programa de Licenciatura en Biología, Universidad Nacional Autónoma de México, Ciudad de México, México. *Both authors contributed equally.
Contacto Dr, Ezequiel M. Fuentes Pananá: (55) 44349663 E-mail: empanana@yahoo.com

Introduction. Gastric carcinoma (GC) is the third cause of death by cancer worldwide. Intestinal and diffuse types of GC initiate within a chronic inflammatory gastritis, therefore GC is considered an inflammatory disease. The main inflammatory risk factor to develop GC is infection with *Helicobacter pylori* (*Hp*) in 90% of cases, although it also expresses the oncoprotein CagA. While, Epstein–Barr virus (EBV) infection has been associated with the 10% of the remaining cases (termed GCaEBV) based on a direct transforming role of EBV. EBV is a lymphotropic virus that persists in memory B cells; up to date, it is not clear how EBV reaches the stomach and whether it triggers an inflammatory effect that cooperates with *Hp* carcinogenesis. Also, we do not know what effect *Hp* has on EBV. Evidence from our laboratory indicates that EBV participates in the development of inflammatory precursor lesions, since only patients co-infected with *Hp* and EBV develop severe inflammatory gastritis that increase the risk of developing GC. In addition, a positive association was also found between high levels of antibodies against the viral structural protein VCA and an increased risk to develop intestinal GC in adults and severe gastritis in children, which suggests a probable cooperation between both pathogens promoting inflammation and tissue damage.
Goal. To analyze if *Hp* promotes the chemotaxis of EBV positive B lymphocytes (EBV+ B lynphocytes), and if *Hp* induces the viral reactivation of EBV in EBV+ B lymphocytes.
Methods. Akata EBV+ GFP+ and EBV+ HS445 B lymphocytes were infected with *Hp* (both strains CagA + and CagA-) and with dead bacteria as negative control. The expression levels of *BZLF1* gene, the master regulator of EBV reactivation, was quantified by RT-PCR and the number of viral copies released into the supernatant by quantitative real-time PCR (RT-qPCR) using standard DNA curves of the EBV *BALF5* gene. Migration assays were performed using conditioned medium (CM) from AGS cells infected with *Hp* (CagA+ and CagA-) as chemotactic factor.
Results. We observed a higher production of viral copies in the direct infection of EBV+ B lymphocytes with *Hp* CagA+ than infection with *Hp* CagA- or death bacteria. In addition, infection with *Hp* CagA+ increased expression of *BZLF1*. The levels of CagA+ vs CagA- expression of BZLF1 are significantly different ($p < 0.05$), as well as the levels of expression of CagA+ vs death CagA+, while the infections of CagA- and death CagA- were not significant different. Likewise, the CM of AGS infection with *Hp* CagA- induced the migration of lymphocytes EBV+, although this effect was stronger for lymphocytes stimulated with CM from AGS infected with *Hp* CagA+.
Conclusions. These data fits a model in which infection with Hp CagA+ strains is a chemoattratant of EBV infected B lymphocytes, which in the stomach environment are reactivated, releasing viral particles that could infect the gastric epithelium contributing to local inflammation and tissue damage.

Pasteurella multocida can use human lactoferrin as iron source by binding it to the aldehyde-alcohol-dehydrogenase

Omar Gómez-Arenas, Erasmo Negrete-Abascal, Sergio Vaca-Pacheco
Facultad de Estudios Superiores Iztacala
Av. De Los Barrios #1, Los Reyes Iztacala, Tlalnepantla, Edo. De Méx. CP.5409, Tel. 56231254
omar.retro14@gmail.com

Pasteurella multocida is a Gram-negative anaerobic facultative cocobacillus bacterium that causes diseases in different animals inducing economic losses to the animal industry. This microorganism expresses different virulence factors given it the capability to infect a wide range of hosts. A limiting to infect a host is the low available iron free concentration. To handle with this limiting, microorganisms express proteins on bacterial surface able to interact with iron-associated molecules such as: hemo-proteins, lactoferrin or transferrin. Some *P. multocida* strains increase the expression of an outer membrane protein of approximately 95 kDa, in the absence of free iron. This protein binds to human lactoferrin as was seen by dot-blot; it was recognized by immune sera of *P. multocida* infected animals and was identified as aldehyde alcohol dehydrogenase by mass spectrometry. *P. multocida* strain ATCC 43020 was able to use human lactoferrin as iron source when it was grown in the presence of 2´2´-dipyridyl as iron chelating agent, recovering until 60% of its normal growing; this recover was not observed with the C3 field-isolate *P. multocid*a strain that does not up-express the 95 kDa protein in iron limiting conditions. Capability to use human lactoferrin as iron source by some *P. multocida* strains could allow them to colonize this host and become in a potential zoonotic agent.
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Microbiological and biochemical study of the *Killer toxin* (*K*1) of *Saccharomyces cerevisiae* in acidic environments

Jaqueline González-Barragán*[1]; Verónica Morales-Tlalpan[2]; Jorge Luis Chávez-Servín[2]; Roberto Ferriz-Martínez[2]; Teresa García-Gasca2 & Carlos Saldaña[1,2]
* Bachelor in Biology. [1]Membrane Biophysics Lab. Basic and Applied Microbiology Unit. [2] Faculty of Natural Science. Autonomus University of Querétaro. C.P. 76140.
carlos.saldana@uaq.mx

In *Saccharomyces cerevisiae* have been reserch different RNA fragments with viral origin, the expression of these fractions give the yeast several characteristics (Killer strains or K^+), among which is the ability to produce protein toxins. They have 3 main types of virus (ScV-M1, M2 and M28) have been described, and each of them codes for a specific killer toxin (*K*1, *K*2 and *K*28). The existence of these toxins provides the ability to kill strains that do not present viral RNA (sensitive or K^- strains) in their cytoplasm.

Currently, different proteins have been considered as potential molecular targets for toxins. However, there is controversy about the interaction with each of them and their molecular targets. In this work, we address the study of the possible interactions between the *K*1 toxin and one of its molecular targets: the TOK1 potassium channel present in *S. cerevisiae*, as well as a study of the stability of the pH acid toxin. In the current project we studied this effect under two aspects: 1) Experimental, directly measuring the killer effect at different pH's, quantitatively determining its effect and its duration, as well as the effect on survival through spectrophotometric studies. 2) The structural stability by stochastic methods that allow to study the stability of the Killer toxin at different pH by means of the *in sillico* modeling of the toxin and even with its molecular target.

During the anaerobic fermentation phase, in our work group we detected and quantified the Killer effect with greater efficiency at acid pH. Our results indicate that the effect of the toxin is irreversible at acidic pH. This which allows generating a new line research of the toxin, also studying its stability at different pH and maintaining an experimental correlate of its capacity to kill the sensitive strains.

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Functional diversity of ant escamolera gut microbiota unveils key adaptations for its survival in a semiarid ecosystem

Jorge Gonzalez-Escobara, Alicia Grajales-Lagunesb, and Ana Paulina Barba de la Rosaa
aDepartment of Molecular Biology, Instituto Potosino de Investigación Científica y Tecnológica A.C., S.L.P., México and bDepartment of Faculty of Chemistry, Universidad Autónoma de San Luis, S.L.P., México.
*apbarba@ipicyt.edu.mx
Palabras clave: Liometopum apiculatum, 16S rRNA. Microbiome

Introduction. Insects have been adapted to diverse trophic habitats through their feeding behavior by evolving several mechanisms for plants components digestion as well as cope with plant defenses 1. Many insects possess a complex gut microbiota but their potential role in the adaptation of its host´s ecosystem is poorly understood2. *Liometopum apiculatum* (escamolera) ant is an important species of edible insect that contributes to the food supply for humans3. They are distributed in arid and semi-arid ecosystems, which has a relative shortage of resources food compared to tropical ecosystems. However, the escamolera ant has reach an ecological balance through diverse symbiotic interactions to survive4, involving mechanisms that are still unknown.
Therefore, we explore functional contribution of escamolera ant gut microbiota regarding the microbial importance in insect nutritional ecology and in potential biotechnological applications.
Materials and methods.The characterization of ant gut microbiome (larval and adult) was carried out through 16S rRNA gene sequencing and culturomic analysis (MALDI-TOF-MS). Bacterial enzyme activity was assayed with API ZYM system.

Result and discussion

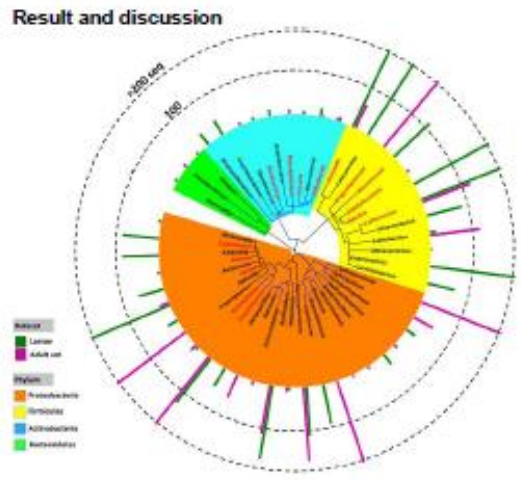

Fig. 1. Phylogenetic tree of bacteria found in the metagenomic analysis and bacteria isolated by culturomics. ML analysis was constructed by 16S rRNA gene sequences alignment. Distances were estimated using the Kimura 2-parameter model in Mega 6.0. Bacteria identified by Maldi-Biotiping are showed in red letters.

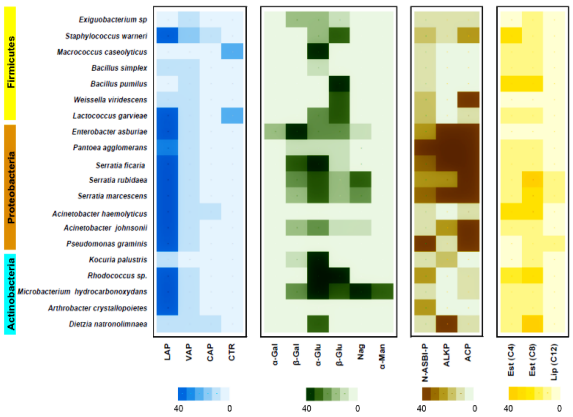

Fig 2. Bacterial enzymatic activities using the API ZYM system (catalytic activity range of 0 to 40 nanomoles). Leucine aminopeptidase (LAP), valine aminopeptidase (VAP), cysteine aminopeptidase (CAP), α-chymotrypsin (CTR), α-galactosidases (α-Gal), β-galactosidases (β-Gal), α-glucosidases, (α-Glu), β-lucosidases (β-Glu), N-acetyl-β-glucosaminidase (Nag), α-mannosidases (α-Man), phosphohydrolase (N-ASBI-P), acid phosphatase (ACP), alkaline phosphatase (ALKP), esterase C4, C8 and C12 (Est C4, Est C8 and Est C12).

Conclusion. Our study provides insights into the functional capabilities of the microbiota of escamolera ant. The results highlight the important contribution of bacteria in the feeding and surviving of ants, and simultaneously, expose a promising source of diverse enzymes for potential biotechnological applications.
Acknowledgments. JLGE thanks to CONACYT fellow 386100.

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Norovirus and rotavirus gastroenteritis severity in children

Guadalupe González-Ochoa, Jesús Adelmo Rosas Anaya, Lilian Flores-Mendoza, Patricia Tamez-Guerra.

[1]Universidad de Sonora, División de Ciencias e Ingeniería, Departamento de Ciencias Químico Biológicas y Agropecuarias, Navojoa, Sonora, México, 85880.

Enteric **v**iral infections are one of the diarrhea- mobility and mortality leading causes in children worldwide. This study aimed to determine the incidence, the gastroenteritis severity and the molecular genotyping of rotavirus, norovirus, astrovirus and enteric adenovirus as gastroenteritis viral agents among children up to five years old. The gastroenteritis severity was determined by using the Ruuska and Vesikari score, whereas the enteric-viruses detection and genotyping were done by retrotranscriptase-PCR and sequence analysis. Rotavirus had the major incidence (18/115); nevertheless, norovirus had the highest severe gastroenteritis score (13±3 points). Results indicated that 56% (10/18) of the detected rotavirus strains were genotype G12P[8], 50% (4/8) of norovirus were GII.4 and 25% (2/8) were genotype GI.8. Out of the sapovirus positive samples, 30% (2/6) were genotyped as GI.I and GII.I. To our knowledge, this is the first sapovirus GI.1 genotype reported in Mexico. Sixty percent of the astrovirus strains (3/5) were genotype HAstV-2, and 20% (1/5) was genotype HAstV-6. One out of the two adenovirus strains was identified as human adenovirus subgenera C type 6. The diarrhea severity reduction in children provides evidence that the rotavirus vaccination program in the northwest of Mexico has been efficient, even among children infected by rotavirus emergent strains G12. The implication resulting by the norovirus becoming the severe gastroenteritis leading pathogen in children, followed by rotavirus and other enteric viruses, is discussed.

Biosynthesis and functions of zwitterionic membrane lipids and their hydroxylated derivatives in *Burkholderia cenocepacia* J2315

Napoleón González-Silva, Otto Geiger, Juan J. Valdez-Alarcón, Maritza R. García-García, Yesica S. González-Torres, Blanca Z. Villagran-de la Mora and Alma D. Ibarra-Ramírez
Universidad de Guadalajara, Centro Universitario de los Altos, Departamento de Ciencias Pecuarias y Agrícolas. Carretera a Yahualica, Km. 7.5 Tepatitlán de Morelos, Jalisco, México. Tel.: 01 (378) 78 280 33 ext. 56810, E-mail: napoleon.gonzalez@cualtos.udg.mx

The Gram-negative bacterium *Burkholderia cenocepacia* J2315 (*Bc*J2315) is a member of the Burkholderia cepacia complex (BCC), a subgroup of important virulent opportunistic pathogens, that infects humans with cystic fibrosis, chronic granulomatous disease, and immunosuppressed individuals, causing a high mortality rate. *Bc*J2315 is multi-resistant to various antibiotics. Zwitterionic lipids constitute the majority of lipids in this bacterium and they comprise ornithine lipids (OLs) and phosphatidylethanolamine (PE) as well as their hydroxylated derivatives, namely 2-OH-OLs (OLs hydroxylated at the C2 of the esterified acyl), NL1 (OLs hydroxylated at the C2 of the amidated acyl), NL2 (OLs hydroxylated at the C2 of both acyls) and 2-OH-PE (PE hydroxylated at the C2 of the acyl esterified at the *sn*-2 position). The presence of hydroxylated versions of membrane lipids has been associated with resistance to stress. In the last two decades most genes involved in standard and hydroxylated OLs biosynthesis have been identified. However, the functions and biosynthesis of hydroxylated OL versions have not been fully elucidated in *Bc*J2315. The genes *olsB* and *pssA* participate in the first step of the biosynthesis of the OLs and of PE, respectively. This research aims to generate a double mutant in *olsB* and *pssA* in *Bc*J2315 that would be unable to synthesize zwitterionic membrane lipids or their hydroxylated derivatives, in order to determine whether these lipids are essential or if they contribute to tolerate stress. Previously a mutant deficient in the *olsB* gene was generated and it does not synthesize any form of OLs. This mutant will be used as genetic background to delete the *pssA* gene (*bcal2355*) and thus generate the double mutant. So far we have made the genetic construct to delete the *bcal2355* gene and the same gene was cloned into an expression vector. Sequencing analysis confirmed that expression vector carries the DNA fragment coding for BCAL2355. The results of this project will clarify the roles of zwitterionic membrane lipids in *Burkholderia* species.

Oxidative stress actives the persistence of *M. tuberculosis* in the human macrophage through WhiB3

Silvia Guzmán-Beltrán[1], Omar Morales-Barrientos y Martha Torres Rojas
Instituto Nacional de Enfermedades Respiratorias Ismael Cosio Villegas, Calzada Tlalpan No. 4502, CDMX. 14080, México. Tel 54871734. guzman.silvia@gmail.com[1]

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (Mtb). An essential characteristic of Mtb is the ability to survive within human macrophages. Consequently, it is vital to know the interaction between host cell and Mtb. In this regard, it has been described that the physiological state of the macrophage as; the levels of O_2, CO, redox state and pH may influence the metabolism and persistence of Mtb in the cell host. It is also known that Mtb responds to microenvironment conditions through various mycobacterial transcriptional regulators. WhiB3 is a transcriptional regulator with redox response domain that responds to the gas level and low pH. This regulator activates the dormant state, promoting the persistence in the host cells. Despite its structure, the effect of oxidative stress on the expression and function of WhiB3 is unknown. *The objective* of this study was determined if the oxidant microenvironment from human macrophages modifies the WhiB3 expression and its function. *Methodology:* Monocytes were obtained from peripheral blood of healthy volunteers, differentiated into macrophages for 7 days and pre-treated with hydrogen peroxide (H_2O_2) and a generator system of superoxide anion ($O_2^{\bullet-}$). Subsequently, macrophages were infected at 1:10 of multiplicity of infection for 1 h, non-phagocytized bacteria were removed and cells were incubated for 2 h in the presence of H_2O_2 and $O_2^{\bullet-}$. We quantified the expression of the antioxidant enzymes, WhiB3 and genes regulated by WhiB3 by RT-PCR. *Results*: The expression of antioxidant enzymes and WhiB3 increased in Mtb in the context of infection compared to mycobacteria cultured in vitro. Interestingly, the WhiB3 function also increased in these conditions. Conclusions: The oxidant state in human macrophages induced the WhiB3 expression, this is a mycobacterial transcriptional regulator that may activate the synthesis of reserve lipids produced to survive in the latency state in its host, which allows its persistence for long periods of time.

Proteomic analysis of *Escherichia Coli* Detergent-Resistant Membranes (DRM): An insight of membrane raft content.

José Enrique Guzman-Flores, Lidia Steinemann-Hernández, Claudia Rodríguez-Rangel, Luis Eugenio González de la Vara, Marina Gavilanes-Ruiz, Adrián Fernando Alvarez and Dimitris Georgellis
Departamento de Genética Molecular, Instituto de Fisiología Celular. Universidad Nacional Autónoma de México. México, DF. 04510, Tel: 56225738 E-mail: eguzman@email.ifc.unam.mx

Membrane microdomains or membrane rafts play notable roles to sub-compartmentalize different cellular processes by organizing membrane components (such as proteins and lipids), allowing the correct functionality of processes. These structures are described in animal, plant and recently in bacterial cells. Detergent-Resistant Membranes (DRM) are the most common approach for identify the content of those microdomains. In this work, we analyze DRMs of *Escherichia coli* by mass spectrometry to give a general sight about protein content of membrane microdomains. This technique led to us identify 79 proteins enriched in DRM fractions accordingly these proteins could be residing in membrane rafts. Analysis by WB of DRM fractions and subcellular location in whole cells by fluorescence microscopy were performed to validate these results.

Analysis of biofilm growth condition by microscopy and production of indole-3-acetic acid production by *Azospirillum brasilense.*

Acatitla- Jácome Iris, Víctor I. Viruega-Góngora, Ma. Luisa Xiqui-Vazquez Beatriz E. Baca, Alberto Ramírez-Mata.
[1] Centro de Investigaciones en Ciencias Microbiológicas, Benemérita Universidad Autónoma de Puebla. Edif. 103J. Puebla, México. alberto.ramirez @correo.buap.mx

Azospirillum brasilense is one of the most important Plant Growth-Promoting Rhizobacteria (PGPR). Elucidation of biofilm structure is a prerequisite to understand the growth of cells within the extracellular matrix sheltering the cells, in the plant growth – promoting rhizobacteria (PGPR) *Azospirillum brasilense* and its role in colonization to plants of agronomic importance. In this study, we used fluorescence and confocal laser scanning microscopy (CLSM) microscopies for study the spatial-temporal formation of the biofilm formation under abiotic conditions. The goal of this work was to describe the process of biofilm formation and determine the role of some cell structures, which might constitute the biofilm matrix. Different methodologies such as crystal violet staining, fluorescence microscopy, confocal microscopy, Western blot and high performance liquid chromatography (HPLC) to determine the auxin phytohormone indol-3-acetic acid (IAA) production were used. For all experiments *A. brasilense* was grown under biofilm formation conditions and samples were taken and analyzed at different time.

The observation of biofilm growth conditions by CLSM revealed the polar flagella and expolysaccharides (EPS) as cells structures promoted cells adhesion and constituted the matrix of biofilm. However polar flagellum flagellin was depolymerized and eliminated after three days of biofilm growth, However the EPS concentration was raised, suggesting that the first step in biofilm formation is an attachment, a step where the bacterium first contacts a surface. To overcome surface repulsion, *A. brasilense* utilizes flagellar-mediated swimming motility that can promote the adherence of bacteria to the surface. Then, we suggested that EPS promote a firmly attachment to the abiotic surface, DNAe was also observed indicated that flagellum, EPS and DNAe were components of biofilm matrix. After five days of growth twice types of colonies small and normal size were observed. Under this conditions when tryptophan is added to culture medium the production of the IAA was detected, the phytohormone that the bacterium use as part of colonization strategy and phytostimulation to interact with plants. Knowledge on biofilm (formation mechanisms and its components) will lead to an improvement in the understanding of bacteria-plant interaction.

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Determination of factors that define the tropism of astrovirus in established cell lines.

Guzmán-Ruiz Leticia, Aguilar-Hernández Nayeli, Arias Carlos F.
Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, UNAM, Cuernavaca, Morelos, México, C.P. 62210, e-mail: lguzman@ibt.unam.mx

Human astroviruses (HAstVs) are one of the most important causes of viral diarrhea in young children, immunocompromised patients, and elderly. The astrovirus genome consists of a single-stranded positive-sense RNA. They are non-enveloped icosahedral viruses of about 35 nm in diameter. Classic HAstVs belong to eight different serotypes that have been associated with human intestinal infection (nonbacterial gastroenteritis). Nowadays, it has been reported that other types of astrovirus can cause neurological syndromes and encephalitis in immunocompromised patients.
Different serotypes of human astrovirus have shown a different efficiency of infection in different cell lines. In our laboratory it has been shown that different cell lines are differentially permissive for astrovirus infection. The susceptibility or resistance of the cells could be determined at different levels of the replication cycle, such as cell binding and entry, protein synthesis, genome replication, virion assembly, or virus cell release. The cellular permissiveness to infection has been shown to be different among different cell lines, since Caco-2 cells were infected by HAstV more efficiently than MA104 cells. With this previous evidence, in this work we wanted to determine the steps in the HAstV replication cycle that determines the different tropism in Caco-2 and MA104 cell lines. There are two possibilities to explain this difference; the first is that not all the cells in the MA104 cell population can be infected by astrovirus, and the second is that all the cells get infected, but MA104 cells produce fewer amounts of virus. We found that at least in the Caco-2 cell line, all the cells in a culture were infected by astrovirus, unlike the MA104 cell line, were just 11 percent of the cells showed to be permissive. These results indicate that only a subpopulation of MA104 cells is permissive to astrovirus infection. On the other hand, we established that astrovirus-infected Caco-2 cells produce 124 and 105 viruses per infected cell for HAstV serotypes 1 and 8, respectively. On the other hand, MA104 cells produced 36 and 25 viral particles per cell infected with HAstV serotypes 1 and 8, respectively. Thus, in Caco-2 cells the viral progeny produced is about 3 times higher than in MA104 cells for HAstV-1, whereas for serotype 8 this difference is about 4 times. This suggests that the permissiveness of the virus in MA104 cells are being limited by the 11% of cells infected, as well as by a post-entry step of the virus cycle replication. To further characterize the replication of HAstV in Caco-2 and MA104 cells, we carried out one-step growth cycle curves in both cell lines. Our results suggest that one of the reasons for the different permissivity of the cells to HAstV could be due to alterations in the early steps of virus infection.

Keywords: Astrovirus, tropism, cell lines, permissive.

Isolation and characterization of bacteria resistant to cobalt

César Díaz Pérez[1], Blanca Estela Gómez Luna[1], Rafael Alejandro Veloz García[1], Lenin Sánchez Calderón[2], Mauricio Nahuam Chávez Avilés[3], Patricia Castro Moreno[4]. [2]Doctorado en Ciencias Básicas. Universidad Autónoma de Zacatecas. [3]Instituto Tecnológico Superior de Ciudad Hidalgo. [4]Unidad de Biomedicina. FES- Iztacala-UNAM. [1]Departamento de Ingeniería Agroindustrial. Campus Celaya-Salvatierra. Universidad de Guanajuato. Priv. de Arteaga s/n, Col. Centro, C.P. 38900. Salvatierra, Gto. cdp276@gmail.com

Zacatecas has approximately 450 years in the mining activity, due to this, there are a plenty of sites contaminated with heavy metals (HMs). In the last decades, several works have been reported high levels of HMs. The Bote‘s dam is one of these sites since one of the oldest pits flows into it, due to this, it has been found high concentrations of HMs as silver, lead, zinc, copper, cadmium. In this work we isolated and characterized resistant bacterial that were able to withstand different concentrations of HMs. Soil samples were taken from five different points of the dam. Eighty-six different bacterial isolates were chosen based on their morphology. The isolates were confronted in solid medium with arsenic, chromium, cobalt, copper and zinc; It was observed that eighteen microbial isolates were resistant to two or more HMs. From the results obtained it was found that the isolates S401-1A and S401-B had a multi-resistance in all the metals confronted. A second confrontation was made to several concentrations of Co in liquid medium, and the optical density was measured. The data indicated that in the presence of Co at 0.05 mM, has a beneficial effect for the isolate S401-B, since it has an increase in its growth compared to the control without metal, in the other hand the isolate S114-1A that had a decrease in its growth. Genomic DNA extraction was performed and the 16S ribosomal gene was amplified. A Molecular phylogeny reconstructing of the isolates was done. The phylogeny tree showed that bacterial isolates belongs to the genus Pseudomonas. It can be concluded that in the Bote‘s dam from Zacatecas, there are bacteria that have been evolved to resistant multiple HMs.

Assessing squash root microbiome along a historical aridity gradient

Cristóbal Hernández Álvarez [1] , Felipe García Oliva [2] , Daniel Piñero Dalmau [3] , Rocío Cruz Ortega [4] , Shamayim Martínez Sánchez [1] , Hugo Barajas de la Torre [1] , Luis D. Alcaraz [1]*
[1] Laboratorio de Genómica Ambiental. Departamento de Biología Celular, Facultad de Ciencias. [2] Instituto de Investigaciones en Ecosistemas y Sustentabilidad. [3]Departamento de Ecología Funcional, [4] Departamento de Ecología Evolutiva, Instituto de Ecología. Universidad Nacional Autónoma de México.
* lalcaraz@ciencias.unam.mx | http://genomica.fciencias.unam.mx

Climate change may have adverse effects in the agricultural sector, which could be affected by the declining of temperate climates and the growing of drier areas. Arid zones have a microbial diversity able to survive under dryness conditions, that could be selected for the plants that live under drought stress to improve their health. With this in mind, we studied squash (*Cucurbita pepo*) root microbiome in localities with different levels of historical aridity in local source samples and a common garden experiment.The common garden experiment considered soil inocula, fertilized inocula, and sterilized controls. To assess the bacterial diversity, V3-V4 regions of the 16S rRNA gene from soil, rhizosphere and endosphere samples, were amplified and massively sequenced. Taxonomic assignment of OTUs, allowed us to identify taxa from each condition. For all samples, Proteobacteria was the most abundant phylum with an increasing abundance of rhizosphere, root endosphere, and the sterilized controls. The dominance of Proteobacteria resulted in a reduction of diversity, which established a decreasing diversity gradient from soil to root endosphere. Comparisons of the relative abundance of phyla between arid and humid samples exhibited the highest abundance of Actinobacteria in arid samples. Beta diversity analyses showed differences related to the level of aridity and can be explained by pH and aridity index. With the common garden experiment, we were able to find plant phenotypic variables related to bacterial communities. Finally, we found OTUs differentially abundant between arid and humid climates, the taxa from arid environments with the capacity to promote plant growth could play a role facing conditions related to the climatic change in agriculture.

Effect of antimicrobial activity of hexanic and ethanolic extracts of *Lippia graveolens* against the *Salmonella enterica*

María Susana Hernández-Hernández[1], Alma Teresa Miranda-Quiroz[2], Agustín Iván Cabezas-Aguilar[2], Abel Hernandez-Martinez[1], Silvia Liliana Bucio-López[2]. Universidad Tecnológica de Morelia, Av. Vicepresidente Pino Suarez 750, 4ta Etapa Ciudad Industrial, 582000, Michoacán de Ocampo, Mich. E.mail: almatmq@hotmail.com
***[1]Ingeniería Bioquímica, Instituto Tecnológico Superior de Ciudad Hidalgo [2] Ingeniería en Biotecnología, Universidad Tecnológica de Morelia*; almatmq@hotmail.com**

Key words: *Lippia graveolens, Salmonella enterica,* antimicrobial

The consumption of food contaminated with bacteria has become a risk to the health of humans, with enterobacteria being one of the main threats for this sector. The *Salmonella enterica* belongs to the family *Enterobacteriaceae* (Barreto *et al*., 2016), and is one of the main bacteria causing food poisoning worldwide because it is transmitted to humans through food of animal origin, causing diseases such as salmonellosis, typhoid fever and gastroenteritis (Jurado *et al*., 2010). Added to this, the indiscriminate use of antibiotics has generated that the microorganisms acquire resistance to them, becoming a public health problem (Quesada *et al*, 2016). Due to the above at present, they are currently looking for alternative treatments with natural substances for the control of diseases caused by *Salmonella enterica* (Yaguana, 2015). The mexican oregano (*Lippia graveolens*) is an aromatic plant belonging to the *Verbenacea* family (Flores-Martínez *et al*., 2016), in addition to having a common culinary use, they are attributed to antibacterial, antifungal, antiparasitic, antimicrobial and antioxidant properties (Reyes-Jurado *et al*., 2014). *L. graveolens* contains compounds such as thymol and carvacrol, to which antimicrobial activity is attributed, in addition to m-cymene, γ-terpinene, caryophyllene, caryophyllene oxide, trans-α-bergamotene, eugenol, and α-bergamotene (Tellez *et al*., 2017). The objective of the present work was to evaluate the antimicrobial activity of the hexanic and ethanolic extracts of *Lippia graveolens* against the bacterium *Salmonella enterica*, by the agar diffusion technique. As controls were used ethanol and hexane without extract, the treatments were carried out in triplicate in concentrations of 10, 5 and 1%, were incubated for 24 hours at a temperature of 37 °C. The results of the ethanolic extract showed an average inhibition halo of 9 mm in the control, 25 mm in the 10%, 23 mm in the 5% and 17 in the 1%, in comparison with the hexane extract which presented an average inhibition halo of 6 mm in the control, 23 in the 10%, 19 in the 5% and 14 in the 1%. The above suggests that the extract of *Lippia graveolens* is capable of inhibiting the growth of *Salmonella enterica*, so it could be an alternative treatment to combat this bacterium.

Determination of the regulatory mechanism of the Toxin-Antitoxin system of plasmid pUM505

M.C. Karen Cecilia Hernández Ramírez, D.C. Martha Isela Ramírez Díaz, D.C. Víctor Meza Carmen. Instituto de investigaciones Químico Biológicas. ED. B-3, Ciudad Universitaria, Morelia, Mich. C.P. 58030. marthaisela_ramirez@hotmail.com

The Toxin-Antitoxin (TA) systems are related to important cellular processes and their regulation by the antitoxin is fundamental for their activity. Recently, in our working group it was determined that the *pum*A and *pum*B genes of plasmid pUM505 encode a TA system that participates in the stability of plasmids in *Escherichia coli*. The protein encoded by *pum*A decreases the growth of *E. coli* BL21 when it is overexpressed, suggesting that it corresponds to the toxin module of the TA system PumAB. Additionally, the presence of the *pum*A gene in *P. aeruginosa* PAO1 transformants increases its virulence in mice, an effect that is neutralized by the presence of the *pum*B gene. These results suggest that the protein encoded by *pum*A acts as a toxin that can be regulated by its cognate antitoxin. Analysis of the secondary structure of the PumB protein showed the presence of a DNA-binding HTH domain suggesting that the PumB protein can regulate the TA system by binding to the promoter region of the *pum*AB operon. Therefore, it is of interest to characterize the form of regulation of the TA system of pUM505. Recently it was determined that in *P. aeruginosa* PAO1 (pUM505) (native plasmid) and in *E. coli* BL21 (pTrc2A_pumAB) (where the *pum*AB genes were cloned without their promoter and the expression depends on the plac promoter), the transcript levels of the gene *pum*B were superior to *pum*A´s. This result was not expected since both genes are part of an operon and are expressed as a bicistronic mRNA; this result suggested that probably *pum*A mRNA is degraded. In order to determine if the PumB protein affects the stability of *pum*A mRNA, the effect of the addition of PumB *in vitro* on the stability of the mRNA of the *pum*A gene was evaluated by RT-qPCR. The results showed that the level of mRNA of the *pum*A gene decreased in the presence of PumB and that this decrease was influenced by the concentration of the PumB protein used. Suggesting that PumB participates in the degradation of the mRNA of the *pum*A gene. In order to determine if the PumB protein binds to the promoter region of the *pum*AB operon, the promoter-operator region of *pum*A was amplified and incubated with increasing concentrations of the PumB protein, to later analyze changes in electrophoretic mobility by means of EMSA tests. The results showed a change in the mobility of the band corresponding to the promoter-operator region of *pum*A in the presence of PumB, indicating that the PumB protein physically binds to the promoter-operator region of the PumAB operon in a specific manner, suggesting the regulation of the expression of the *pum*AB operon by the PumB antitoxin.

Influenzavirus A(H1N1)pdm09 enhances the adhesion of *A. fumigatus* resting conidia to the alveolar epithelial cell line A549.

Fernando Hernández-Sánchez, Carlos Alberto Vanegas-Torres, Luis Angel Pérez-Moreno, Gabriel Palma Cortés, Carlos Cabello Gutiérrez*.
Departamento de Virología y Micología, Instituto Nacional de Enfermedades Respiratorias –Ismael Cosío Villegas". Calzada de Tlalpan 4502, Col. Sección XVI, Deleg. Tlalpan. CDMX. México. C.P. 14080. *carloscginer@gmail.com

Aspergillus is commonly considered as a non-pathogenic oportunisitc colonizator of lungs, however it can easily become invasive when patients have an ongoing, or a recently relieved infection with *Influenzavirus*. Severe pulmonary invasive aspergillosis (PIA) secondary to influenza gives rise to a death risk five times higher than the viral illness itself, and represents an epidemiological threat in the context of future influenza outbreak scenarios. Whether influenza increases the adhesion of *Aspergillus* conidia to alveolar epithelium, favoring invasiveness, has not been adressed to date. Two complementary lines of evidence, using the A549 cell line (type II pneumocytes) as a model, suggest that adhesion molecules may play a role in the influenza-facilitated fungal dissemination. On one hand, it is known that adhesion and internalization of *Aspergillus fumigatus* conidia is mediated by the physicalinteraction with the integrin alpha 5 / beta 1 heterodimer (Int-α5/β1) or with E-cadherin. On the other hand, both Int-α5/β1 and E-cadherin have been proven to be up-regulated after influenza infection of cells. In this work we infected monolayers of A549 epithelia with AH1N1 pdm09 influenzavirus to evaluate its effect on the adhesion of *A. fumigatus* resting conidia, and on the expression of the above mentioned adhesion molecules. We hypothesized that A549 cells infected with AH1N1pdm09 will show an increment in the *A. fumigatus* adhesion to the monolayer surface, and that this increment will be associated with the expression level of Int-α5/β1 or E-cadherin.

Our results showed, in the first place, that A549 cells are susceptible to infection with influenza virus, reaching a near complete infectivity using M.O.I. of 1, and without loss of the monolayer integrity at 24 h post-infection, as revealed by immunocytochemistry. Under these conditions, the number of fungal colonies detected (directly related to the adhesion of resting conidia by plate assay) in the presence of virus increased 50% at 24 h. A similar trend was observed for integrin alpha 5, and integrin beta 1 at the protein level (observed independently by Western Blot); accompanied by a three fold increase of the mRNA for their respective genes ITGA5 and ITGB1 (assayed by qPCR). E-cadherin showed no change.

Conclussions: Infection of alveolar epitelial cells with AH1N1pdm09 favors the adhesion of *A. fumigatus* resting conidia, which correlates with the augmented expression of Int-α5/β1.

Isolation and characterization of bacteria with plant growth promotion rizobacteria (PGPR) associated to native maize of a region of the state of Guerrero.

Zaida Asunción Hernández-Martínez[1], Gerardo Emmanuel Caballero-Reyes[1], Jorge Antonio Tzec-Interían[1], María Inés Torres-Martínez[1], Ofelia Ferrera-Rodríguez[1], Randy Ortiz-Castro[1]*.
[1]Instituto de Ecología A.C., Red de Estudios Moleculares Avanzados, Laboratorio de Microbiología Ambiental y Fitopatología, Carretera Antigua a Coatepec 351, El Haya, C.P. 91070, Xalapa, Veracruz, México.
*Corresponding author: randy.ortiz@inecol.mx

Abstract

Maize is the second grain with the highest production at a global level and the food base of several countries. Mexico is the center of origin and diversity of corn. The accelerated population growth, the low technification of the Mexican countryside and the attack by phytopathogens that affect the crops, demand a greater production of this grain. In this crop, a high dose of chemical fertilization is used, an aspect that represents a high cost and risk investment for farmers in the region. The traditional cultivation of corn has significantly reduced the quality of the soil by excessive use of agrochemicals and fertilizers have demanded the search for development of new environmentally friendly alternatives like bioproducts based on microorganisms such as the use of plant growth promoting rhizobacteria (PGPRs), it is an important alternative since they produce secondary metabolites that promoted the plant growth through phytohormones production like auxin, gibberellins, citokinin and other regulators; antagonistic effect (antibiotics and lytic enzymes) and can also induce systemic resistance in plants (ISR). In the present study, we carry out the isolation and characterization of bacteria with PGPR activity associated to native corn by using an *in vitro* system Arabidopsis-bacterium to identified isolates with potential plant growth promotion activity. Of a total of 301 bacterial isolates at least 133 endophytic isolates, increased the plant growth promotion around the 43% in relation with Arabidopsis seedlings without inoculation, most notably among them the isolates En1.2_17, En1.3_07, En1.3_10 and En2.2_12 showed the plant growth promoting activity inducing the formation of lateral roots and fresh weight. Analyzing the expression of *DR5::uidA* Arabidopsis seedlings we found that the mechanism of plant growth induction involves the participation of an auxin-like response related with the promotion of plant growth in the response to the bacteria.

SEARCH, AMPLIFICATION AND CLONING OF THE GENE *csrA* IN *Bacillus licheniformis* M2-7

Juanita Jacobo Valerio; Isabel Villa Morales; Estefany Isabel Higuera López; Miguel Á. Rodríguez; Augusto Rojas Aparicio; Yanet Romero Ramírez, Gerardo Huerta Beristain
Laboratory of Biotechnology, Autonomous University of Guerrero, Mexico. C.p. 39070. Tel: 7471020121. Email: hbgerardo @gmail.com

Introduction: The carbon storage regulator or Csr, is a global regulation system that controls bacterial gene expression post-transcriptionally, constituted by two fundamental units, CsrA and CsrB[1]. It has been seen only that in *Bacillus subtillis* the inactivation of csrA causes greater mobility of the bacteria, without any other cellular effect[2]. The bacterium *Bacillus licheniformis* M2-7 was isolated from the municipality of San Marcos, Guerrero[3], it has been shown that this strain is a degrader of hydrocarbons such as oil, gasoline and burnt oil, It was also shown to promote plant growth and showed anti-fungal activity against mango phytopathogenic fungi[3]; so when making modifications it is possible to potentiate these characteristics.

Objectives: To identify the system in Csr in *Bacillus licheniformis* M2-7 and to generate a molecular tool that will serve in the medium term to generate a mutant in the csrA gene.

Materials and Methods: The bioinformatic analysis was carried out using the sequence of the *csrA* gene of *Bacillus subtilis* ATCC 19217 as a template, later the comparison with the genome of *Bacillus licheniformis* was made, and the design of primers was carried out. For the standardization of the csrA gene amplification it was subjected to a temperature gradient (52, 55, 58.60 and 62 ° C), after obtaining the csrA amplicon the cloning was carried out in *E. coli* DH5α was subsequently carried out the selection of transformants to extract and purify the plasmid DNA and finally the confirmation by restriction reaction.

Results: It was possible to identify and amplify the csrA gene with a length of 773 bp in *Bacillus licheniformis* M2-7 at the temperature of 58 ° C which was optimal for the alignment, cloning and confirmation was performed in *E.coli* DH5α.

Conclusion: The construction of plasmid pJIER17 was carried out, to subsequently carry out a mutant of the csrA gene in *Bacillus licheniformis* M2-7.

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Assessment of protein fractions culture supernatant of *Mycobacterium bovis* in interferon gamma release assays by ELIspot

Laura Jaramillo Meza1*, Fernando Díaz Otero1, Romina Alejandra Ortega Armenta1
Laura Hernández Andrade1, Marco Antonio Santillán Flores1
1CENID-Microbiología Animal INIFAP. Carretera Federal México-Toluca Km. 15.5, Col. Palo Alto Cuajimalpa, Ciudad de México, CP 05110, Tel. 52 55 38718700,
E mail: jaramillo_meza@yahoo.com.mx

Interferon gamma (IFN-γ) is one of the most critical effector molecules to the establishment of a cell-mediated immune response required for control of *Mycobacterium bovis* infection, so its evaluation have been considered a biomarker of disease. The EliSpot assay allows for the identification of a wide range of cell populations based upon their cell function and their production of specific immune molecules. In tuberculosis diagnosis, this assay has been used as a research tool to detect cell populations that are releasing IFN-γ following exposure to mycobacterial antigens. The aim of this study was to evaluate the ability of inducing IFN-γ fractions of the protein extract from the culture filtrate of *M. bovis* separated by isoelectric focusing in ELIspot assays. For which, *M. bovis* strain AN5 was grown in Dorset-Henley liquid medium at 37°C for 6 weeks; subsequently, cultures were filtered obtaining free-bacteria medium. Filtered constituent proteins were precipitated with ammonium sulfate, then preparative liquid-phase isoelectric focusing was used for the separation of culture filtrate protein (CFP). This procedure resolved CFP into 20 fractions with a pI range of 2.59 to 12.9. These fractions were subjected to immunological analysis by ELIspot; for which, were separated PBMC from 24 cows tuberculin reactors and 22 non-reactors.
The cells culture were also stimulated with bovine PPD and avian PPD, ESAT-6 and CFP-10 antigens, as well as with pokeweed mitogen and the no-antigen control. The quantification of reaction points was performed using an automatic counter ImmunoSpot 3.2. IFN-γ produced with different antigens in whole blood cultures were also evaluated by ELISA. A Kruskal-Wallis test was performed to analyze the results obtained between groups and the Mann Whitney test was used to compare the different antigens. The analysis indicated statistical difference among bovine PPD, the CFPE, ESAT-6 and the fractions 1, 2, 3, 4, 9, 11, 14, 15, 16 and 19. However, greater specificity was determined to bovine PPD itself, the ESAT-6 protein fractions 1, 2, 3, 4, 17 and 19 for the group of reactors cows. By electrophoretic analysis of the fractions, it was determined that the MPB70 and MP83 proteins are located in fractions 3 and 4, which correspond to specific proteins of *M. bovis*. Highlight study the reactivity of cow's reactors to antigens fractions 5, 6 and 7, in which heat shock proteins and enzymatic activity proteins are located. According to results, the fractions 1, 2, 3, 4 and 17 showed greater specificity in assays release of IFN-γ assessed by ELISA and ELIspot, by which are suitable for the purpose searched of disease diagnosis.

Área: Microbiología y Virología

Evaluation of the effect of trypsin on the biofilm-forming capabilities of commensal and infective isolates of *Staphylococcus epidermidis*.

Sergio Martínez-García, Sandra Rodríguez-Martínez, Mario E. Cancino-Diaz, Janet Jan-Roblero, and Juan C. Cancino-Diaz.
Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas, Department of Microbiology, Laboratory of Immunomicrobiology, Mexico City, México.
Head of group: Dr. Juan Carlos Cancino-Diaz
Carpio y Plan de Ayala S/N, Col. Santo Tomas, Delegación Miguel Hidalgo, 11340, Mexico City, Mexico. Phone: 57-29-63-00 Ext: 62464. Email: jccancinodiaz@hotmail.com

Coagulase-negative staphylococci (CoNS) are the main agents responsible for nosocomial infections and represent the most common source of infections associated with medical devices. Biofilms confer resistance to antibiotics and to the host immune response, and they represent the main mechanism underpinning CoNS infections. Biofilm formation has been found to be a differential discriminant biomarker between clinical and commensal isolates. Clinical studies have reported that most (ca. 50–60%) CoNS isolates are biofilm. The remaining clinical isolates (ca. 50–40%) are considered to be non-biofilm-forming, and these have not been extensively studied. It has been shown that non-biofilm-forming clinical *S. epidermidis* isolates are able to induce biofilms after treatment with trypsin through processing of the accumulation-associated protein (Aap) and promoting biofilm-formation, suggesting that *S. epidermidis* biofilms are composed of innate (formed without adding proteases to culture media) and protease-induced biofilms. In this study, the trypsin biofilm-induction capacity was evaluated in a large number of non-biofilm-forming *S. epidermidis* isolates (*n*=133) in order to support this mechanism and to establish the importance of total-biofilms (innate plus induced). *S. epidermidis* isolates from ocular infections (OI; *n*=24), prosthesis joint infections (PJI; *n*=64) and healthy skin (HS; *n*=100) were screened for innate-biofilm formation according to the Christensen‘s method. By comparing clinical (43.2%) vs commensal (17%) innate-biofilm producers, significant differences were found ($p<0.0001$). Meanwhile, non-biofilm-forming isolates were treated with trypsin (0.2, 2 and 20 □g/mL), and biofilm formation was evaluated by the same method. A significant increase of trypsin-induced biofilm isolates was observed in commensal isolates (74.7%) compared to innate-biofilm isolates (17%). In contrast, clinical isolates increased from 43.2% (innate-biofilm) to 52% (trypsin-induced biofilm). The comparison between clinical and commensal total-biofilm producers (innate-biofilm plus trypsin-induced biofilm) yielded no significant differences ($p=0.392$). The result was the same when different samples were tested (OI vs HS and PJI vs HS). Morphology of the trypsin-induced biofilms was evaluated by SEM, which resembles an innate biofilm morphology. It was found that trypsin-induced biofilms are able to protect indwelling cells from antibiotics, such as ciprofloxacin. The *icaA/aap* genotype was determined by PCR in non-biofilm-forming isolates. The genotype *icaA-/aap+* was associated with the trypsin-induced biofilm phenotype. Non-biofilm-forming commensal *S. epidermidis* isolates (*n*=116) from different body sites (head, nostril, axillae and conjunctiva) formed trypsin-induced biofilms in 70 isolates (50.3%), indicating that commensal isolates have a high capacity for biofilm formation by trypsin.

In conclusion, the present study provides evidence that trypsin is capable of inducing biofilm production in non-biofilm-forming commensal *S. epidermidis* isolates with the *icaA-/aap+* genotype. Our results also demonstrate that there is no significant difference in totalbiofilms when comparing clinical and commensal isolates, suggesting that total-biofilms are not a discriminant biomarker.

CHARACTERIZATION OF A NOVEL CIPROFLOXACIN RESISTANCE PROTEIN PLASMID ENCODED, CrpP

Víctor M. Chávez-Jacobo, Jesús Silva-Sánchez and Martha Isela Ramírez-Díaz*
Instituto de Investigaciones Químico-Biológicas, UMSNH, Ed. B-3. Ciudad Universitaria. CP.58030, Morelia Mich. *marthaisela_ramirez@hotmail.com

Ciprofloxacin (CIP) is a synthetic chemical agent and among the most commonly prescribed antimicrobial because of their broad spectrum of action. Extensive clinical and agricultural use of CIP has led to high rates of resistance to these agents among pathogenic microbes; however, CIP is the most active quinolone against Pseudomonas aeruginosa and is used to treat infections generated by this bacterium. The most common mechanism of resistance to CIP is the mutation of chromosomal genes encoding DNA gyrase and/or topoisomerase IV. Changes in the expression of efflux pumps and porin proteins are also common CIP resistance mechanisms in bacteria. Plasmid mediated quinolone resistance (PMQR) include the Qnr protein, which prevents CIP binding to target proteins, efflux pumps QepA and OqxAB. Moreover, modified aminoglycoside N-6´-acetyltransferase (AAC(6´)-Ib-cr promotes CIP resistance.

The pUM505 plasmid, isolated from a clinical P. aeruginosa strain, confers resistance to CIP. In silico analysis, performed to identify CIP resistance gene, showed that the 65 amino acid product encoded by the orf131 gene in pUM505 displays 40% amino acid identity to the Mycobacterium smegmatis aminoglycoside phosphotransferase. We cloned orf131 (renamed CrpP, for ciprofloxacin resistance protein, plasmid encoded) into the pUCP20 shuttle vector and the recombinant plasmid pUC_CrpP conferred resistance to CIP. Using coupled enzymatic analysis, we determined that the activity of CrpP on CIP is ATP dependent, suggesting that CIP may undergo phosphorylation. Using liquid chromatography-tandem mass spectrometry, we also showed that CIP was phosphorylated prior to its degradation. Then, our findings conclude that CrpP represents a new mechanism of CIP resistance in P. aeruginosa.

Partial characterization of a lipoprotein from coliphage mEp021 involved in Superinfection Exclusion

Martínez Soto Carlos E., Martínez Peñafiel Eva, Kameyama Kawabe Luis Y. Departamento de Genética y Biología Molecular. CINVESTAV-Unidad Zacatenco. Av. Instituto Politécnico Nacional 2508, Col. San Pedro Zacatenco, Delegación Gustavo A. Madero, CDMX, Código Postal 07360. Apartado Postal: 14-740, 07000 CDMX, Tel: +52 (55) 5747 3800 luisk@cinvestav.mx

SUMMARY

A new family of nonlambdoid phages (mEp) was classified into a unique immunity group (Immunity group I). None of the phages belonging to this immunity group were able to grow in a lysogenic *Escherichia coli* W3110 harboring the prophage mEp021, presumably due to its repressor. However, from mEp021 genome sequence, we identified an open reading frame (ORF82) which encodes for a 72 amino acid polypeptide (Lpp^{mEp021}), which we propose is responsible of superinfection exclusion (Sie). In addition, protein BLASTp showed that Lpp^{mEp021} has a lipobox consensus sequence and shares around 15% identity with other Sie related proteins of phages HK022, phi80, N15, mEp167, Sh6 and T5. The amino acid sequence of Lpp^{mEp021} presented a signal peptide characteristic of excretion and transmembrane proteins. On the other hand, we localized *in silico* two possible promoters upstream of ORF82, and one possible terminator downstream of this gene. The expression of the cloned ORF82 into pKQV4 expression vector was able to exclude mEp021 in a *E. coli* strain W3110. Interestingly overexpression of ORF82 showed reduced Sie for mEp021 in a strain W3110. Sie for mEp021 was inhibited in ORF82 expressing mutant strains where, *lam*B, *mal*F and *mal*G genes were knocked out (Keio collection), indicating that the OM protein LamB and the IM proteins MalF and MalG are necessary for the exclusion mediated by Lpp^{mEp021}. Adsorption of mEp021 was shown to be inhibited by Lpp^{mEp021}, indicating a possible interference of the primary receptor recognition and the normal adsorption to the cell. Interestingly, none of the 13 different bacteriophages tested from immunity group I were excluded by LppmEp021 suggesting the they require a different receptor than the one from mEp021(OmpA). Lpp^{mEp021} was localized in the inner and outer membrane of the bacteria by Western Blot, suggesting that Sie mediated by Lpp^{mEp021} is being carried out at a complete membranes level, where it interacts with membrane proteins such as LamB, MalF and MalG, inhibiting mEp021 adsorption and therefor its infection.

Study of essential genes in *Pseudomonas aeruginosa*.

Enrique Martínez-Carranza[1], Luis David Alcaráz-Peraza[2], Luis Servín-González[1] Gloria Soberón-Chávez[1]*. [1] Departamento de Biología Molecular y Biotecnología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México. [2] Facultad de Ciencias, Universidad Nacional Autónoma de México. [1]*Edificio C, Laboratorio 110, Ciudad Universitaria, Ciudad de México. gloria@biomedicas.unam.mx. Tel. 55-56229201.

Pseudomonas aeruginosa is a highly adaptable bacterium that thrives in a broad range of ecological niches, and in humans it is an important opportunistic pathogen in compromised individuals. With the aim of comparing several *P. aeruginosa* genomes and discerning the genetic requirements essential for growth in different conditions and culture media, recently a number of comparative genomics studies have been performed in *P. aeruginosa* strains. The increased availability of genome sequences has provided the basis for comprehensive understanding of organisms at the molecular level and high-throughput genomic approaches such as transposon sequencing have been used to identify essential genes. In order to understand phylogenetically the genomic information has been classified in three major classes. The core genome, which includes all genes responsible for the basic aspects of the biology of a species and its major phenotypic traits. By contrast, dispensable genes or accessory genome, contribute to the species diversity and might encode supplementary biochemical pathways and functions that are not essential for bacterial growth but which confer selective advantages, such as adaptation to different niches, antibiotic resistance, or colonization of a new host, is acquired by horizontal gene transfer (HGT). Given that these genes are not necessary for survival or maintenance of the species, they can also be deleted from the genome. The pan-genome is the global gene repertoire of a bacterial ~~-s~~pecies": core genome + dispensable genome. Until now has been defined that the essential genes are in the core genome, however, it has been postulated that the essential genes not always are part of the core genome. Several studies have proposed a certain amount of essential genes in *P. aeruginosa* with several approaches and in several culture conditions. Moreover several works consider that essential genes are variable among species and among strains. In this work we evaluate the conservation of those candidates for essential genes of *P. aeruginosa* proposed in different studies in the strains PAO1 and PA14, and the conservation of the candidates for general essential genes (in six culture conditions) of *P. aeruginosa* and in the close relative *Azotobacter vinnelandii.* With our analysis we can propose that the conservation of essential genes is greater than expected and that the postulation of candidate essential genes based on high-throughput genomic approaches must be carefully reviewed and confirmed with other approaches as deletion of single genes

Obtention of a *ctpF* knockout mutant in *Mycobacterium tuberculosis*: a potential target of attenuation

Milena Maya Hoyos[1], Marisol Ocampo Cifuentes[2], Carlos Yesid Soto[1]*
[1]Grupo Bioquímica y Biología Molecular de las Micobacterias, BBMM, Departamento de Química, Facultad de Ciencias, Universidad Nacional de Colombia, Carrera 30 # 45-03, Bogotá, Colombia.
[2] Fundación Instituto de Inmunología de Colombia (FIDIC), Carrera 50 # 26-20, Bogotá, Colombia.
*Corresponding author e-mail address: cysotoo@unal.edu.co (Carlos Y. Soto)
Chemistry Department, Faculty of Sciences, Universidad Nacional de Colombia, Carrera 30 # 45-03, Ciudad Universitaria, Postal code 111321, Bogotá, Colombia.
Phone number: +57-1-3165000 ext. 14445; Fax number: +57-1-3165220

Abstract:
Tuberculosis (TB) is an infectious disease caused by the acid-fast bacillus *Mycobacterium tuberculosis* (*Mtb*). According to the World Health Organization (WHO), TB caused 10.4 million new cases and 1.7 million deaths in 2016. The incidence of TB has increased due to the existence of multidrug and extensively resistant (MDR and XDR) mycobacterial strains, *Mtb*-HIV coinfection and the low efficacy of Bacille Calmette Guerin (BCG) vaccine. The identification of alternative targets involved in the virulence of *Mtb* is key for the rational design of attenuated *Mtb* strains. Some studies have suggested that membrane proteins are pivotal for the tubercle bacillus viability. Indeed, the deletion of P-type ATPases produces impaired transport of cations across the *Mtb* plasma membrane affecting the response to toxic substances, virulence and viability of the *Mtb*.

In the present work, an *Mtb* H37Rv *ctpF* null mutant was obtained by recombineering techniques. Genotypic analysis confirmed the identity of the Δ*ctpF* mutant strain. Furthermore, the cytochemical staining of *Mtb* cells with neutral red suggested that *ctpF* could be associated with the *Mtb* virulence. This promissory result has to be confirmed *in vitro* and animal models, in order to postulate the *ctpF* gene as an *Mtb* attenuation target with vaccine potential.

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Biological traps to recruit plant associated microbiomes, in multiple types of soils

Gerardo Mejía [1], Luis D. Alcaraz [1*]
[1] Laboratorio de Genómica Ambiental. Departamento de Biología Celular, Facultad de Ciencias, Universidad Nacional Autónoma de México. 04510, Ciudad Universitaria, Ciudad de México,
* lalcaraz@ciencias.unam.mx | http://genomica.fciencias.unam.mx

Microorganisms found at the roots are able to enhance, disease or being neutral in the development and growth of plants. Our approach for recruiting growth-promoting rhizosphere microorganisms used multiple microbiome (soil) sources and evaluate the plant phenotype (60 days after germination). We established a screening system to capture growth-promoting microbiomes using tomato (*Solanum lycopersicum*) in a common garden experiment under hydroponic conditions. We used hydroponic cultures to subject plants of *S. lycopersicum* to the presence of different soil sources to collect the root interacting microbiomes. Multiple sterile and nutrient controls were included to test those variables. Multiple plant phenotypic variables are being evaluated: amount of chlorophyll, length, weight dry, weight wet, stem diameter and leaf surface. We found differences related to stem diameter, length and leaf surface.
We selected the plants with significant differences to extract rhizospheric metagenomic DNA and then used both 16S rRNA gene amplicons and shotgun sequencing to describe taxonomic and metabolic profiles. We are currently analyzing metagenomic data to understand the taxa and coding genes responsible for plant growth promotion. The main advantage of our scrutiny system is the possibility to discard environmental variables like soil source, weather, water availability, and plant genotype effects in a common-garden setup and focus on the microbiome effects in the plant host. Finally, our system is being refined to be a useful tool in the study of plant-microorganism interactions.

Filamentous Fungi Cell Wall PIR Proteins: Diversity, Function and Application

Paul Montaño-Silva, Benjamín Rodríguez-Garay and Jorge Verdín
Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco (CIATEJ);
Zapopan, Jalisco, Mexico 45019. (33) 3345 5200 ext 2103, jverdin@ciatej.mx.

Proteins with internal repeats (PIR) are cell wall resident proteins (CWP) highly conserved in yeasts. The internal repetitive units they contain consist of eight highly conserved amino acids (S, Q, D, G, Q, Q, A). Additionally, they may contain a characteristic four cysteine motif at the C–terminus. PIR proteins can be found covalently attached to the cell wall either through an alkali-labile ester bond between a glutamine residue of the repetitive units and β1,3-glucans, or via a disulfide bond between the C-terminal cysteine domain and other CWPs. *Saccharomyces cerevisiae* PIR proteins are involved in the maintenance of the cell wall structure and it has been found that confer heat shock resistance. Because of their easy and controllable detachment from the wall, these proteins have been widely used in yeasts as molecular anchors for cell surface protein display, a technology that allows the introduction of new activities and other assets to target microorganisms. Despite two putative PIR proteins have been bioinformatically identified in *Neurospora crassa*, XP_957648.1 (PIR-1) and XP_958614.3 (PIR-2), the diversity and function of PIR proteins in filamentous fungi have not been studied. In this work, the diversity of fungal PIR proteins were analyzed and their function explored utilizing *N. crassa* PIR-1 and PIR-2 as models. 375 PIR-motif bearing proteins were identified, which clustered in two main groups. The first one matched to the classical yeasts PIR proteins (four cysteine motifs at the C-terminus, no GPI signal). The second one included PIR proteins from yeasts and filamentous fungi. Most of them contain GPI signals and diversified cysteine-rich motifs (from 3 up to 8 cysteine residues) located in any region of the protein. *N. crassa* PIR-1 and PIR-2 belong to the second cluster. In addition to two repetitive domains, PIR-1 contains an N-terminal five-cysteine motif. In contrast, PIR-2 only contains five repetitive domains and no cysteine motifs. In order to explore the function of Nc PIR proteins, two knock-out (KO) strains, *Δpir-1* (FGSC# 16451*)* and *Δpir-2* (FGSC# 17985) were phenotypically analyzed. Unexpectedly, the growth rate of both strains was higher (*Δpir-1:* 2.88±0.6 mm/h; *Δpir-2*: 2.7±0.6 mm/h) than that of the WT strain (2.38±0.6 mm/h). Moreover, branching rates did not show any significative difference between *Δpir-1* and WT strains (2.33±0.84 br/500 µm; 3.06±0.73 br/500 µm, respectively). However, *Δpir-2* strain showed a branching rate (3.70±0.98 br/500 µm) significantly higher than that of the other two strains. To determine the cell wall integrity, Calcofluor white (CFW) assays were performed. *N. crassa* WT (FGSC# 1901), showed a minimal inhibitory CFW concentration of 1mg/ml, while both KO strains were inhibited by 0.75 mg/ml CFW. Taken together, these results suggest that both PIR proteins are important for the cell wall structure. We hypothesize that the lack of any of them lead to a more flexible cell wall that allows a higher growth rate. This work was supported by a grant from SENER-CONACYT –Sustentibilidad Energética" (245750).

Epinephrine and norepinephrine induce *Actinobacillus seminis* biofilm dispersion

Juan Fernando Montes-García[1], Pamela Bautista-Betancourt[1], Sergio Vaca-Pacheco[1] Candelario Vazquez Cruz[2], Erasmo Negrete-Abascal[1]
[1]Facultad de Estudios Superiores Iztacala, UNAM. Av. De Los Barrios 1, Los Reyes Iztacala, 54090 Tlalnepantla, Méx. 2Centro de Investigaciones en Ciencias Microbiológicas, Instituto de Ciencias, BUAP, Apdo. Postal 1622, Puebla 72560, México.

Actinobacillus seminis is a Gram negative bacterium member of the Pasteurellaceae family considered as autochthonous of reproductive organs of ovine, but also is the causal agent of epididymitis and of low rates of fertility mainly in immature sexually ovines. Pathogenesis of *A. seminis* is not well known and its virulence factors are scarcely characterized. It has been suggested its ascension from prepuce towards epididymis after an increase of luteinizing or follicle-stimulating hormones during puberty. *A. seminis* is able to form biofilm when growing in brain hearth infusion medium (BHI) without agitation and at 37 ºC at least 48 h. In this work the effect of epinephrine (E) and norepinephrine (NE) in *A. seminis* biofilm formation was studied. The addition of 50 µg/mL of E or N into BHI did not produce significant changes in the growth rate of an *A. seminis* reference strain and two field isolates tested; however, those hormones induced a lower biofilm formation of the three strains. This effect was more noticeable with NE than with E and also in one of the field isolates. Stress hormones could induce the dispersion of the *A. seminis* biofilm and thus promote the infection of surround tissues in ovine reproductive organs.
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Characterization functional of riboswitches T-box in Firmicutes and the other gram-postive bacteria.

Nataly Morales Galeana and Enrique Merino Pérez. Instituto de Biotecnología, UNAM. Av. Universidad 2001, Col. Chamilpa, C.P. 62210. Cuernavaca, Morelos. Tel. 0155 - 329629 (ext. 288) natalymg@ibt.unam.mx, merino@ibt.unam.mx.

In Firmicutes and the other gram-positive bacteria, the riboswitch T-box regulate the transcription a variety of genes related to metabolism of amino acids. The riboswitch T-box sense charged and uncharged tRNAs and can bind to a tRNA in two sites, the first through the interaction between codon-anticodon and the second through the sequence 5´UGGN3´ which binds to the sequence 5´NCCA3´ only if tRNA is uncharged. Whether tRNA is uncharged the pairing promotes formation of the antiterminator and the transcription continue while with charged tRNA the structure terminator is more stable, and the transcription is inactive1. Bioinformatics analyses can predict riboswitches T-box in the bacterial genomes, because of sequences and structural characteristics are conserved. In our lab, the fine analyses allowed the identification of riboswitches T-box with additional characteristics and these are enlisted below:

1.-**Riboswitch T-box *leu*S of *Carboxydothermus hydrogenoformans* that contain a tRNA^{ala} gene embedded within stem III.**[2] The conservation in sequence of riboswitch and tRNA and your presence in the other Clostridia suggest both elements in this riboswitch are functional. We hypothesize that this tRNA is removing by processing of RNAsa P and/or RNAsa E.

2.-**Riboswitch T- box tandem in 5´UTR of operon *trp*E in *Bacillus cereus*.2** The regulation of biosynthesis of tryptophan in *B. cereus* seems to be different to *Bacillus subtilis* and *Escherichia coli.* We believe the arrangement of riboswitches in tandem is due to the need to for respond adequately to concentrations of tryptophan. We hypothesize both subunits are capable to respond independently a low concentration of charged tRNA^{trp}. We pretend characterize the functionality of riboswitches mentioned previously and we are using anti-sRNAs for obtain strains with low concentrations of charged tRNAs. We constructed fusions the riboswitches with gene reporter *lac*Z and integrated it in *B. subtilis* for sense your activity regulatory through FDG assay.

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Regulation of *E2348C_1013* by GrlA in enteropathogenic *Escherichia coli*

Álvaro Damián Morales Ibarra, Emilio Cadena Guinto, Ramón Cervantes Rivera and José Luis Puente García.
Instituto de Biotecnología de la Universidad Nacional Autónoma de México. Av. Universidad #2001 Colonia Chamilpa. C.P. 62210. Tel. 777-329-1627. puente@ibt.unam.mx

Enteropathogenic *Escherichia coli* (EPEC) is a human diarrheal pathogen mostly of children 6-24 months old. EPEC belongs to the A/E (Attaching/Effacing) family along with enterohemorrhagic *Escherichia coli* (EHEC) and the mouse pathogen *Citrobacter rodentium*. A/E pathogens attach intimately to the enterocyte and efface the microvilli. The genes required for A/E lesion formation are encoded within the Locus of Enterocyte Effacement (LEE) pathogenicity island. The global regulator H-NS represses LEE gene expression forming nucleo-repressor complexes with the LEE promoters. The LEE-encoded regulator Ler, which binds to DNA regions in the vicinity of the LEE promoters, antagonizes H-NS-mediated repression. GrlA and GrlR are also LEE-encoded regulators. GrlA is a positive regulator of *ler* expression that binds to an ATGT sequence motif located between the -35 and -10 *ler* promoter boxes; in contrast, GrlR homodimers repress LEE gene expression by interacting with GrlA, thus preventing it from binding to the *ler* promoter. PerC, an EAF plasmid-encoded regulator, can also activate the *ler* gene depending on the growth conditions. The promoter of the *E2348C_1013* (*E1013*) gene, which encodes a hypothetical protein, shares a significant identity with the *ler* promoter, particularly in the spacer region around the ATGT motif. Complementation experiments in *E. coli* K12 with plasmids expressing the different EPEC regulators and transcriptional fusions between different fragments of the *E1013* promoter region and the promoterless *cat* gene, showed that the activation of the *E1013* promoter was GrlA-dependent, suggesting a role of this gene in virulence. In addition, we determined that the minimum regulatory region sustaining GrlA-mediated activation of *E1013* spans from positions -43 to +26 with respect to the transcriptional start site and that PerC has no effect in its activation. Moreover, a mutation in the A base of the ATGT sequence drastically reduced *E1013* promoter activation by GrlA, confirming the importance of this sequence motif. We also observed that the non-coding untranslated region (UTR) downregulates *E1013* expression in an H-NS independent manner. These results are allowing us to look into variations of the GrlA mechanism of action at different promoters.

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Potential of colonization from fungal endophyte isolated from *Eichhornia crassipes* on bean plants.

[1]Adán Topiltzin Morales-Vargas, [1] Blanca Estela Gómez Luna, [2]Varinia López-Ramírez and [1]Luis Ángel Hernández Romero
[1]Ingeniería Agroindustrial, División de Ciencias de la Salud e Ingenierías Universidad de Guanajuato, Campus Celaya-Salvatierra. [2]Coordinacion de Ingeniería Bioquímica. Instituto Tecnológico superior de Irapuato. Carretera Irapuato-Silao km. 12.5 C.P. 36821Irapuato, Guanajuato
adan.tops@gmail.com

Plants respond to abiotic stress through a complex signaling system involving the perception of signal transduction by means of signaling pathways, followed by genetic and physiological responses. Plants are commonly colonized by a wide range of endophytic microorganisms, such as bacteria and fungi. Recently it has been demonstrated that the response to stress in plants can be mediated by microbial symbiotic associations. These microorganisms are diverse and have an impact on plant communities through the fitness of some process such as: increased tolerance to biotic and abiotic stress, increased biomass or decrease in water consumption, etc. Endophytic community present in a plant is influenced by various factors such as geographic location, weather patterns, plant physiology and tissue specificity. This work aims evaluate the potential of fungal endophytic isolates from *Eichhornia crassipes* to grow endophytically in crop plants, we used 23 different fungal isolates to systematically test their potential to colonize bean plants. Molecular identification of fungal endophytes was based on sequence analysis of ITS1 genomic region. For each strain, a stock suspension was prepared in sterile solution 0.1% Tween 80 and 30% sugar containing about 1×10^6 conidia ml^{-1} and 40 ml of this suspension was applied to bean seeds for 2 min, control treatment received the same amount of sterile solution applied in the same manner. Six to ten seeds of beans were used for each strain and placed in sterile soil to evaluate the germination and growth of the plant. 20 days after inoculation, plant colonization by different fungal strains was determined through re-isolation of the fungus from surface-sterilized of leaves using a microbiological method. Five of the fungal isolates were able to colonize bean plants. The results showed that bean plants. maintains a symbiotic association with these fungal endophytes. It remains an open question about if these fungal endophytic are related with the tolerance to stress. Nevertheless, we conclude that these fungal endophytes should be considered with a promising potential to increase stress tolerance on bean plants

Characterization of plant growth promoting rhizobacterium isolated from saline environments of the Chihuahuan Desert.

Claudia Estefania Navarro Muñoz[1], Juan Ángel Ayala Rodríguez[2], José López Bucio[2], Jorge Sáenz Mata[1].
[1]Laboratorio de Ecología Microbiana, Facultad de ciencias biológicas, Universidad Juárez del Estado de Durango. Av. Universidad S/N Fracc. Filadelfia, C.P. 35010 Gómez Palacio, Durango, Mexico.[2]Instituto de investigaciones Quimico-Biologicas, Universidad Michoacana de San Nicolás de Hidalgo, Edificio A‘, Ciudad Universitaria, C.P. 58030 Morelia, Michoacán, México.

Key words: PGPR, saline environments, *Arabidopsis thaliana*, halotolerance.

The bacterium present in the rhizosphere that have the ability to promote plant growth are called PGPR (acronym in English for growth of plants that promote rhizobacterium) and their mechanisms of action can be indirect and direct action. Within the direct mechanisms are nitrogen fixation, phosphate solubilization, production of siderophores, indole acetic acid, cytokinins and gibberellins for the purpose of resource acquisition.

The objective of the present work was to characterize and evaluate the effects of rhizobacterium, isolated from different seven plants in a saline soil, on *Arabidopsis thaliana*. Afterwards the bacteria were isolated and purified in culture media (NFb), (LB), (KB) and (LB) were added with 50 mg of Sodium Chloride. Continue whit the scrutiny to identify the ability of the rhizobacterium to promote plant growth in *Arabidopsis thaliana* as a model organism by planting seeds from the reporter line DR5: Gus in direct contact for 6 days. To understand the effect of this bacterium we were used growth kinetics lines like CycB1: uidA (cellular division), AtExp7: uidA (Expanding cells) and arr5: uidA (elevated by cytokinin). Subsequently, DNA extraction was performed using the CTAB method for sequencing and identification. 180 bacteria were isolated and purified, as a result of the confrontation with *A. thaliana* resulted 16 strains that promote plant growth. The rhizobacterium called Lbecto1p6 was selected by showing a phenotype of growth promotion and also observing the decrease in lateral roots and elongation of the main root compared to the control. A decrease in auxinic response was observed on the fourth and sixth day in contact with the rhizobacterium on the line DR5: uidA, on the line AtExp7: uidA an increase in the expression of expanding cells was observed on the 6th day; In line CycB1: uidA response to cyclin b1 on fourth and sixth day an increase in expression was observed on contact with the rhizobacterium. For this reason, when we have been evaluating the stages of the primordium, it was found that on the 6th day there is a greater difference in the development of the 7 stages. The molecular identification by means of the sequencing of the 16S rRNA gene gave as a result that the rhizobacterium Lbecto1p6 corresponds to Bacillus sp.

Lambda phage display as a model for a ZIKV vaccine

Negrete-Méndez, Honorio; Martínez-Peñafiel, Eva; Kameyama Kawabe, Luis Y.
Departamento de Genética y Biología Molecular. CINVESTAV-Unidad Zacatenco.
Av. Instituto Politécnico Nacional 2508, Col. San Pedro Zacatenco, Delegación Gustavo A. Madero, CDMX. CP 07360. Apartado Postal: 14-740, 07000.
Tel: +52 (55) 5747 3800
luisk@cinvestav.mx

Summary
Vaccines are considered one of the most significative advances in public health because they achieve the reduction of the mortality and morbidity of a large number of infectious diseases. Vaccine production can be expensive and time consuming.
Therefore, advances in genetic engineering have opened the door for novel approaches based on the selection of specific antigens that accelerate the design and reduce the costs. These engineered vaccines can be administrated in the form of peptides, vectors or virus like particles (VLPs), avoiding the use of attenuated or inactive pathogens. One of these designs is based on Phage display, a technology that allows to display peptides in bacteriophages capsids, has shown a high potential for the development of fast and cost-effective vaccines.
This work describes the construction of a phage display system to produce a vaccine against zika virus (ZIKV). We used the domain III of the envelope protein (EDIII) as antigenic epitope of zika, and fused it to the gpD capsid protein of phage λ. This fused protein (D-EDIII) was overexpressed and purified in a bacterial system, and then co-assembled at low concentration on the phage capsid (Western-blot data).
However, electronic micrographs indicate that this protein generates destabilization of the viral capsid, so the phage was unable to assemble properly or withstand the capsid pressure by the genome packaging.
At this moment, we are working to optimise the assembly of phage λ capsid with the fusion protein, in order to display enough fusion protein to achieve an adequate immune response.

Analysis of the regulation and function of chaperone-usher fimbrial operons in *Citrobacter rodentium.*

Ortiz-Jiménez Stephanie, Caballero-Flores Gustavo and Puente José Luis.
Departamento de Microbiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, México. Av. Universidad 2001, Col. Chamilpa. Cuernavaca, Mor., 62210. puente@ibt.unam.mx

Citrobacter rodentium is a specific mouse pathogen that causes transmissible murine colonic hyperplasia. It belongs to a family of enteric pathogens that colonize the host gastrointestinal tract producing the attaching and effacing (A/E) lesion on the epithelial cell surface. This family includes the human pathogens enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) for which there is no animal model of disease, making of the *C. rodentium*-mouse infection model an excellent *in vivo* model to study the pathogenesis of this bacterial family. The initial adherence of these pathogens to the host enterocytes is often mediated by filamentous structures called fimbriae. The *C. rodentium* genome carries 19 fimbrial operons, of which 13 belong to the chaperone-usher (C/U) family; however, there is limited knowledge about these operons, and only the role of two of them during infection has been studied: *kfcHGFEDC* and *gcfFGABCDE* encode the KFC and GCF fimbriae, respectively.

The transcriptional activity of the C/U fimbrial operon *ROD_11771-ROD_11781,* herein named *crf1,* was assessed using a transcriptional fusion of its putative regulatory region to the promoterless reporter *cat* gen. The activity of this fusion was evaluated in the wild-type strain (DBS100) and in the Δ*hns* and Δ*stpA* mutants under different growth conditions. No activity was detected in the wild-type strain, but a significant increase in activity was observed in the absence of H-NS or StpA, suggesting that these global regulators play a negative role in the expression of *crf1*. To identify *cis-acting* regulatory elements a series of fusions carrying shorter fragments of the upstream putative regulatory region was generated, which allowed delimiting the regulatory region between positions -221 a +37 respect to the ATG. The transcriptional start site (TSS) of *crs1* was identified by primer extension at position -123 with respect to the ATG and the -10 and -35 promoter boxes predicted based on this information. The functionality of these boxes is being assessed by site-directed mutagenesis.

crf1 is an interesting C/U operon because it lacks a gene encoding for the pilus subunit, but contains genes encoding a chaperone and an usher protein. Downstream these two genes, there is an additional gene coding for a hypothetical protein that, according to RT-PCR assays, is co-transcribed as part of the operon. The identity and functional role of this putative protein are being studied using different strategies. Further experiments will also be performed to address the expression and function of this operon during intestinal colonization.

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Human Papillomavirus prevalence in oral cavity from University population

Nidia Gary Pazos Salazar[1], Adriana Morán Torres[1], Susana Téllez Lorenzo[1], Marcela Lizano[2], Joaquín Manzo Merino[2]
1 Laboratorio de Infectología Molecular, Departamento de Microbiología, Facultad de Ciencias Químicas, BUAP
2 Laboratorio de agentes causantes de cáncer. Instituto Nacional de Cancerología
Avenida San Claudio y 14 sur, Edificio FCQ1, Ciudad Universitaria, Col. San Manuel, Puebla, Puebla.

Human Papillomavirus (HPV) infection is considered the sexually transmitted most frequent disease in the world. HPV infects anus genital epithelium, oral cavity and oropharynx. Recently HPV prevalence in oropharynx cancer has increased in a worrying way, reporting 80% of HPV positivity in this cancer. By this reason we determined HPV prevalence in oral cavity and oropharynx from a sexually active population in Benemerita Universidad Autónoma de Puebla. 103 patients were included, and samples from oral cavity and oropharynx were taken using a cytobrush®, immediately a smear was made to realize Papanicolau stain and later the sample was introduced in saline solution for DNA extraction and PCR. For HPV detection a fragment of 450bp corresponding to L1 gen was amplified, the β-globin gen was used as DNA sample integrity and HeLa cells DNA as positive control. HPV genotyping was made amplifying the E6 oncogene from HPV-16, HPV-18 and HPV-6 that has been reported as the most frequent in oral cavity. Results: In eight patients the samples showed no integrity so the analysis was made from 95 patients, 43% were men and 57% women, the majority population (82%) was between 18-25 years of age and the sexual life starts between 15-20 years of age in the 84% of the population studied. Coilocytes were not found in any sample by Papanicolau stain. With respect to HPV detection 43 patients (45.6%) were positive by PCR, mainly in oral cavity, 12 patients were positive in both oral cavity and oropharynx. The genotypes found were; HPV-16 (46%), HPV-18 (18.6%), HPV-6 (0%) and the rest samples were insufficient for genotyping by PCR. The PCR showed to be useful to identify HPV infection in early stages when traditional test as Papanicolau stain cannot detect it.

Rotaviruses associate with extracellular vesicles in MA104 cells

Arianna Pérez Delgado, Susana López, Carlos F. Arias, Pavel Isa*
Instituto de Biotecnología, Universidad Nacional Autónoma de México.
*Av. Universidad 2001, Chamilpa, 62210 Cuernavaca, Morelos. Telef.+525549167743

Rotaviruses, members of the family Reoviridae, are the leading etiologic agent of viral gastroenteritis in infants and young children worldwide. They contain a double stranded RNA genome organized in 11 segments, enclosed in three concentric protein layers. They infect mainly the mature enterocytes of the small intestine villi, however, the majority of the studies have been done in the monkey kidney epithelial cell line MA104. Previous studies have focused on many aspects of the rotavirus replication cycle, describing rotavirus receptors, cell entry routes and intracellular replication and assembly. With respect to rotavirus cell release, it has been believed for long time that rotavirus is liberated from MA104 cells after cell lysis. A recent description that a fraction of the rotavirus progeny is present in the extracellular medium as early as 9 hours post infection, before any cell lysis, has changed this view. A portion of the secreted rotavirus particles migrated to low-density fractions during density gradient centrifugation, suggesting an association with cellular membranes. To characterize this non-lytic pathway for virus exit, we analyzed if the virions could be released associated with extracellular vesicles (EV). All cell types secrete EV, among them, exosomes, apoptotic bodies and microvesicles. Of special interest are the exosomes, which are secreted after fusion of multivesicular bodies with the plasma membrane, and different viruses (hepatitis C and E viruses and HIV) have been found associated with them. To explore the possible association of rotavirus particles with exosomes, we followed the non-lytic secretion of rotavirus particles, observing extracellular virus from 6 to 14 hours after infection. We also observed that a fraction of the secreted viral particles is protected from a protease treatment by membranes since the protection was lost after treatment with Triton-X100, suggesting that the virus could be associated with EV. We purified EVs by differential centrifugation and observed the presence of protected virus mainly in the fraction of microvesicles and exosomes. In addition, we found in the EVs purified from the supernatant of infected MA104 cells the presence of biomarkers for exosomes, suggesting that these vesicles could be associated with the virus. To determine the relationship between the secretion of exosomes and the protected virus fraction, we treated the cell with drugs that inhibit (GW4869) or induce (NH4Cl and bafilomycin A1) the exosome biogenesis, to determine their effect on the protease-protected fraction of the viral progeny with respect to the non-protected fraction. We observed a decrease in the viral titer in cells treated with GW4869 and increase of the virus protected when the cells were incubated with NH4Cl or bafilomycin A1. The purified exosomes were visualized by electron microscopy, and the presence of exosomes and viruses was observed, but not their association. We are currently investigating the effect on the release of viral particles of interfering the synthesis of cellular proteins related to exosome biogenesis. In addition, with a Nanosight we will determine the composition of particles in terms of their size and density. PAPIIT IN210916 grant supports this project.
Keywords: rotavirus, extracellular vesicles; release.

Isolation and purification of *Bacillus subtilis* bacteriophages in soil samples from the state of Nuevo León, Mexico.

Sandra Guadalupe Pérez Martínez, Carlos Eduardo Hernández Luna, Licet Villarreal Treviño and Juan Francisco Contreras Cordero.
Universidad Autónoma de Nuevo León. Pedro de alba S/N, Ciudad Universitaria, San Nicolás de los Garza, Nuevo León. CP 66455. +528183294000 Ext.6432. Email: sandraprm16@gmail.com

Introduction: Bacteriophages are viruses that parasitize bacteria, these play a very important role in the ecology and microbial evolution. It has been shown that bacteriophages are the most abundant entities in the diversity of biomes, despite this, the conditions of terrestrial environments are much more drastic, making it difficult to study them, because the bacteriophage-host relationship is directly affected. The intense cooperative application of isolating, genetics, morphological and physiological techniques helps us to understand from genetic mechanisms, structure and quantification of bacteriophages. This may be an opportunity for scientific research, since it is important to have quick and reliable identification and quantification methods.

Objective: Isolate and purify strains of *Bacillus subtillis* bacteriophages from dry and wet earth samples from the state of Nuevo León.

Material and methods: Wet and dry soil samples were collected from different points in the state of Nuevo Leon, which were processed and analyzed by tests using *Bacillus subtilis* as host, the tests used were direct and enriched isolation, spot test, dilutions in plaque and later characterization of bacteriophages by lytic plates and the diversity between them.

Results: From the positive results, it can be observed a lysis activity and the diversity of bacteriophages due to the morphology of lithic plaques with a range of 0.5 to 3 mm in diameter.

Conclusion: The host *B. subtillis*, represents an excellent model for the isolation of phage from soil samples.

Growth arrest and plasmid copy number control: a proteomic approach

Ángeles Pérez-Oseguera[1], Rosa María Gutierrez[2], Andrés Saralegui[3], Paul Gaytan[4], Sofía Martínez-Absalón[5], Miguel A. Cevallos[1]
Av. Universidad S/N col. Chamilpa CP 62210. Cuernavaca Morelos, México
aperez@ccg.unam.mx Tel 01777 3 2916 90

1 Centro de Ciencias Genómicas-UNAM, Programa de Genómica Evolutiva. 2 Instituto de Biotecnología-UNAM, Departamento de Microbiología Molecular. 3 Instituto de Biotecnología-UNAM, Laboratorio Nacional de Microscopía Avanzada. 4 Instituto de Biotecnología-UNAM, Unidad de Síntesis y de Secuenciación de DNA. 5 Centro de Ciencias Genómicas-UNAM, Programa de Ingeniería Genómica.

The *repABC* operon contains all elements required for stable replication of many alpha-proteobacteria plasmids. RepA and RepB participate in plasmid segregation and in the negative transcriptional regulation of their own operon and *repC* gene encodes the initiator protein and within its coding sequence reside the origin replication of the plasmid.

We constructed two vectors (pSEC-1 and pSEC-1_GFP) whose replication depends on an inducible *repC* gene. The main difference between these vectors is that pSEC-1_GFP contains a gene that constitutively expresses GFP. With the induction of the *repC* gene in *Rhizobium etli* transconjugants carrying pSEC-1, we observed: an evident increase of plasmid copy number (from 1 copy to 86 copies in 18 hrs); a detention in the increase in optical density and a 10X reduction of CFU number. However, a LIVE/DEAD analysis shows that cell viability remains basically the same in induced and not induced cells. Also, we don‘t found differences in cell sizes between treatments. A microscopic time-lapse analysis of *Rhizobium etli* transconjugants, carrying pSEC-1_GFP shows that *repC* induction leads to a rapid growth arrest, nonetheless, cells display a continuous augmentation of fluorescence, as a consequence of an increment of plasmid copy number. When the inductor is removed from culture media, washing the cells and re-inoculated them in fresh medium, cell restart to grow. During this process cells show a continuous, but steady plasmid copy number reduction. After repeating this process two consecutive times, plasmid copy-number return to its original number (1 - 2 copies per chromosome). These observations suggest that growth arrest is a mechanism to deal with an excess of plasmid copies.

To obtain a general picture of the mechanisms involved in this behavior, we made proteomic analyses (LC-MS/MS) of the strain containing pSEC-1, two hrs., four hrs. and 18 hrs., after *repC* induction. Also, we made the same analyses during the recovery phase after washing the cell and re-growing them in fresh medium, three consecutive times (24 hrs. each). Comparative analysis of dynamic proteomic profiles indicates that only three proteins were differentially expressed four hrs after induction, but 18 hrs later, 59 proteins were differentially expressed (19, up and 40 down), specially those involved in amino-acid metabolism, carbohydrate transport and metabolism Posttranslational modification, protein turnover and chaperones. The biological significance of the results will be discussed at the congress.

Insights into the structure of the viral protein genome-linked (VPg) of members of the *Potyvirus* genus

Aldo A. Pérez-Montoya[1], Gustavo Rodríguez-Gómez[2], Mariana Andrade-Medina[1], Laura Silva-Rosales[2] and Mauricio Carrillo-Tripp[1]*
[1]Biomolecular Diversity Laboratory, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional Unidad Monterrey, Nuevo León, México
[2]Departamento de Ingeniería Genética, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional Unidad Irapuato, Guanajuato, México
*e-mail: mauricio.carrillo@cinvestav.mx

Abstract
The viral protein genome-linked, or VPg, is covalently bonded to the 5' end of positive-strand viral RNA. It is believed that it acts as a primer during RNA synthesis in a variety of virus families. At least in a diverse range of potyviruses, VPg has also been implicated in cell-to-cell and systemic movement through the interaction with host proteins. In order to shed light on the molecular mechanisms involved in such a process, here we show advances on the structure determination of the VPg of *Sugarcane Mosaic Virus*, *Tobacco Etch Virus*, *Bean Common Mosaic Virus*, and *Bean Common Mosaic Necrosis Virus*. The methodology we have followed is a combination of theoretical methods for tertiary structure prediction based on sequence and experimental characterization of biophysical properties. Hence, we have implemented a robust procedure to relate *in-silico* models with *in-vitro* thermal stability responses. This methodology is well-suited for the structure determination of other proteins.

Neutralizing activity of anti-M1 antibodies against the Influenza A virus in equines from Nuevo León, Mexico.

Claudia Bernardette Plata Hipólito, Sibilina Cedillo Rosales, Licet Villarreal Treviño, Carlos Eduardo Hernández Luna, Cristina Rodríguez Padilla y Juan Francisco Contreras Cordero.

Universidad Autónoma de Nuevo León. Pedro de alba S/N, Ciudad Universitaria, San Nicolás de los Garza, Nuevo León. CP 66455. +528183294000 Ext.6432. Email: platacb@gmail.com

Introduction: Although the Influenza A virus is widely studied, it does not present a constantly effective prevention against an infection. The genetic variability of the virus, the emergence of new variants and its ability to jump the barrier between species suggest the study of genetically stable elements of the virus. The M1 matrix protein can induce neutralizing antibodies that confer protective immunity against the entire viral particle in animal species. The incidence of Influenza A virus in domestic species represents a risk to human health. This as a potential alternative for the control and prevention of this virus.

Objective: Determine the neutralizing capacity of anti-M1 antibodies against wild strains isolated of equines from Nuevo Leon, Mexico.

Materials and methods: Collection of 4 nasal fluids and sera of trash service horses of Nuevo León was carried out. The presence of the virus and anti-influenza antibodies was carried out by RT-PCR and ELISA type HADAS respectively. Wild strains were propagated in VERO cell line, using the Kentucky/97 vaccine strain as control. Subsequently, viral neutralization assays were performed using anti-M1, anti-vaccine and anti-virus antibodies.

Results: Neutralizing capacity of anti-M1 antibodies with a range of dilutions 1:80 to 1:160 was determined against the vaccine strain and against wild isolates. Observing a superior neutralizing activity of anti-virus antibodies with a range of 1:150 to 1:320 and a lower one with the anti-vaccine antibodies with a range of 1:50 to 1:80.

Conclusions: These results show the neutralizing capacity of anti-M1 antibodies against the complete viral particle, which supports the hypothesis of inducing a heterosubtypic immune response.

Identification and characterization of 3-Oxosphinganine reductase in bacteria

Elva Quiroz-Rocha, Jonathan Padilla-Gómez, Isabel M. López-Lara and Otto Geiger.
Programa de Ecología Genómica, Centro de Ciencias Genómicas, UNAM., Av. Universidad 1001, Col. Chamilpa, Cuernavaca, Mor., México.
Contact: equiroz@ccg.unam.mx

Sphingolipids (SphLs) are essential components of eukaryotic membranes and they play crucial roles in signaling and organizing lipid rafts, as well as in the regulation of cell division, differentiation, migration, programmed cell death, and other processes. Sphingolipid regulation, biosynthesis and function have been studied extensively in eukaryotic cells and some microorganisms (i.e. yeast).
However, few bacteria are known that carry SphLs in their membranes. To date only some Gram-negative bacteria, within the proteobacteria (*Sphingomonas, Acetobacter, myxobacteria*), and the Bacteroidetes (*Bacteroides, Porphyromonas, Sphingobacterium*) are known to possess SphLs, where they functionally replace other bacterial membrane lipids. In eukaryotes, the biosynthesis of SphLs begins with the condensation of serine and a fatty acyl-CoA to form 3-oxosphinganine (stage 1), followed by its reduction to sphinganine by 3-Oxosphinganine reductase (KSR) (stage 2), the acylation to *N*-acylsphinganine (dihydroceramide) (stage 3), and the desaturation to ceramide (stage 4). In stage 5, ceramide is modified with different polar groups to form the great diversity of SphLs. Although the eukaryotic genes involved in the sphingolipid biosynthesis are known, in bacteria little is known on SphL formation. An exception is stage 1, catalyzed by serine palmitoyltransferase, which has been identified and partially characterized from *Sphingomonas*, and *Bacteriovorax.* Although sphinganine (stage 2) is formed in some bacteria, the mechanism is not clear. The objective of this work is to determine which genes participate in the reduction of 3-oxosphiganine to sphinganine in bacteria. *In silico* analyses allowed us to identify in bacteria (*Sphingomonas wittichii*, *Zymomonas mobilis*, *Escherichia coli* BL21(DE3) and *Caulobacter crescentus*) candidates for KSR that might function similarly to KSR previously characterized in *Saccharomyces cerevisiae*. These genes were cloned in an expression vector to induce their expression and analyze their capacity to reduce 3-oxosphinganine to sphinganine. For these purposes an *in vitro* assay was established to measure the formation of sphinganine by using nascently formed ^{14}C-labeled 3-oxosphinganine and reduced versions of pyrimidine nucleotides (NADH or NADPH). Results for potential bacterial KSRs will be presented in the course of this work.

Expression and purification of VP4 structural protein of rotavirus in *E. coli* BL21 bacteria.

Authors: Yuliet Ramirez Cintra, Reyes S. Tamez Guerra, Cristina Rodriguez Padilla, Pablo Zapata Benavidez, Lydia Guadalupe Rivera Morales y Juan Francisco Contreras.
Immunology and Virology Laboratory; Faculty of Biology; Universidad Autónoma de Nuevo León.
Pedro de Alba, Ciudad Universitaria, San Nicolás de los Garza, N.L. C.P. 66455
Tel. 8121219303; email: yuliet.ramirezctr@uanl.edu.mx

The VP4 protein is found on the surface of human rotavirus, whose essential functions are receptor binding and cell penetration. In addition, it is an antigen for the formation of neutralizing antibodies, which makes it an object of study to learn about the protection against licensed vaccines and the development of new vaccines with advanced technologies. The expression and purification of this protein using bacteria such as *E. coli* BL21 is an easy and efficient methodology. However, due to the soluble characteristics of this protein; the purification may not be efficient. Therefore, it is necessary to realize a study of the expression of the protein under different induction conditions to increase its efficiency.

Objective: Obtaining and purifing VP4 protein of human rotavirus using *E. coli* BL21 as an expression model.

Materials and methods: E. coli BL21 was transformed with pGEMX-4T2 which contains the gene coding for the VP4 protein. The transformation was analyzing by with enzymatic restriction using *BambH I* and *Sal I* enzymes and PCR. Expression was performed using 1mM of IPTG after 12 hours of expression at 250 rpm/37°C. The obtained recombinant proteins, accumulated in the bacteria, were solubilized with urea and detergents, analyzed by SDS-PAGE and Western Blot using Wa-antibodies, followed by purification by affinity chromatography.

Results: *E. coli* strain BL21 was transformed with PGEX-4T2-Wa construct. The expression of the protein was performed for 7 hours, the optical density was measured every hour and the exponential growth of the bacteria was observed. In the SDS-PAGE, the expression of the protein was reflected from 3 hours of expression, reaching a higher expression at 7 hours. Protein is still obtained 24 hours later. The Western blot was detected the protein with the VP8*-antibody.

Conclusion: The production of VP4 protein for this method allowed us to get the protein with excellent quality that can use in different process as production of antibodies against VP4 protein.

Characterization of resilient yeast isolated from an agave fermentation process

Bibiana Rios Galicia, Lily X. Zelaya Molina, Juan Lara Aguilera, Oscar Trejo Cerro, Judith Joanna García, Ramon I. Arteaga Garibay
Centro Nacional de Recursos Genéticos CNRG-INIFAP; Tepatitlán de Morelos, Jalisco; email: arteaga.ramon@inifap.gob.mx

Tequila is an alcoholic beverage made from the fermentation and distillation of cooked-hearts of *Agave tequilana* var. azul. Agave plants harbor an indigenous microbial community that initiates the fermentation (Martínez-Rodríguez et al., 2014). During the process, fermentation vessels are maintained to the highest standards of cleanliness. Among the cleaning products, ammonium bifluoride is a common product used to remove surface contaminants; thus, fermentative strains should resist high concentrations of both ethanol and ammonium bifluoride (Aigueperse et al., 2012). The purpose of the research is to isolate endemic agave yeast that resist high ethanol and ammonium bifluoride concentrations. Samples were taken from the agave fermentation process, consisting of raw agave pineapple (PA-1), dead molasses (MM-1) and fermented molasses (MF-1). Yeast colonies where isolated on JAA, YPD and SB solid media. Ammonium bifluoride (Ab) and ethanol susceptibility was assessed by a broth microdilution method. Yeast were cultured in Mueller-Hinton media with different concentrations of Ab and ethanol. Inoculated and uninoculated wells free of ethanol and Ab were taken as controls. Gram positive (*S. aureus*) and Gram negative (*E. coli*) bacteria were incorporated in all the experiments.

There were obtained 14 yeast strains. Five of them belonged to PA-1, two to MM-1 and seven to MF-1. Six isolated yeast were able to grow at high concentrations of ammonium bifluoride. In contrast, all the yeasts tolerated high ethanol concentrations (≥ 7.5%), indicating their big potential as ethanol-producing yeasts. Six yeast isolates are potential inoculants to the fermentation process of agave in the production of tequila for its resistance to ammonium bifluoride-ethanol and its indigenous origin.

Characterization of isolated microorganisms of thermal water of Chignahuapan, Puebla.

David Israel Ríos Vázquez1*, Itzamná Baqueiro Peña2, Candelario Vázquez Cruz3, Ma. Patricia Georgina Sánchez Alonso3, Norma Elena Rojas Ruiz3
1 Licenciatura en Biotecnología. Facultad de Ciencias Biológicas. Benemérita Universidad Autónoma de Puebla
2 Centro de Investigación en Alimentación y Desarrollo, A.C.
3 Instituto de Ciencias, Centro de Investigaciones en Ciencias Microbiológicas.
Benemérita Universidad Autónoma de Puebla. Edificio 103J C. U., Col San Manuel, CP. 71570, Puebla Pue. email: normaelena.rojas.nerr@gmail.com, Tel: 01222 229 55 00 Ext. 3965.

The isolation of microorganisms from extreme environments, as well as the study and use of their biomolecules and their metabolism, represents one of the fields with the greatest potential of biotechnology. In industry it is very important to obtain thermostable macromolecules that are used in processes that are carried out at high temperatures. The objective of this work was the isolation and characterization of the microorganisms that develop in thermal water belonging to the recreational center of Chignahuapan, Puebla.

Samples were taken from the thermal water flows and the pool area. Ten samples were analyzed, the pH and temperature were determined. In Luria Bertani (LB) solid médium 100 µl of each sample was inoculated, the Petri dishes were incubated at 46 °C for 24 hours. Subsequently, the different colonies with different morphologies were counted and isolated. Gram stains of the isolates were performed, as well as mobility tests and production of hydrogen sulfide in SIM medium. Five different strains were selected to perform growth kinetics in seven temperatures (30 °C, 35 °C, 40 °C, 45 °C, 50 °C, 55 °C and 60 °C). DNA was extracted from each of the strains used in the growth kinetics, the gene coding for 16S rRNA was amplified by PCR and sequenced.

In LB solid medium fifty four strains of microorganisms were isolated, the morphologies of the isolates were classified into four groups, three of them were Gram positive and one group of Gram negative bacteria. The microscopic observations showed the presence of microorganisms belonging to the genus *Bacillus sp*. In the mobility tests and production of hydrogen sulfide in SIM medium some of them were positive. In the growth kinetics at different temperatures the five strains tested showed a better development at temperatures in the range from 30 to 45 ºC.

Keywords: Thermal water, microorganisms, Chignahuapan, *Bacillus sp*

Detection of the West Nile Virus NS1 gene in Nuevo León; Mexico

Alejandro José Rodríguez García[1,3], José Santos García[1,3], Licet Villareal Treviño[1,3], Carlos Eduardo Luna Hernández[1,3], Ramiro Ávalos Ramírez[2,4]; Juan Francisco Contreras Cordero[1,3].
Immunology and Virology Laboratory; Faculty of Biology[1], Faculty of Veterinary Medicine and Zootechnician[2]; Universidad Autónoma de Nuevo León[3].
Pedro de Alba, Ciudad Universitaria, San Nicolás de los Garza, N.L. C.P. 66455
Tel. 8121219303; email: alejorod89@hotmail.com; alejandro.grodriguez@uanl.edu.mx

West Nile virus (WNV) is a *Flavivirus* first isolated in Uganda in 1937, since then, it has been distributed throughout the world with the development of nine genetic lineages producing from asymptomatic infection to a neuroinvasive disease infecting different species. Linage 1 is the most scattered across the world and the specific one disseminated in America and Mexico. WNV encodes 10 proteins, three structural and seven non-structural. Nonstructural protein 1 (NS1), crucial for viral replication and immune evasion is produced and located in cytoplasm, plasma membrane and as an extracellular form. This protein stimulates the production of specific immunoglobulins against it, containing the infection. Therefore, this protein is an excellent immunological target for future vaccines and diagnostic tests. This study aims to detect the NS1 protein from the West Nile virus for further diagnostic studies.

Methodology Detection of WNV was carried out amplifying the genes prM/E with primers 212 and 619c previously reported (Lanciotti et al; 2000) supporting our positive control. Later, gene NS1 was amplified through specific primers NS1f and NS1r. These were achieved using Access Reverse-Transcriptase-PCR System (Promega) using manufacturer‘s instructions. Thermal cycler protocol for prM/R was 45 °C for 45 min. for reverse transcription 94 °C for 2 min for inactivation; 40 cycles of 94°C of 30 sec. 55 °C for 1 minute and 68 °C for 2 minutes. With a final extension of 68 °C for 7 min and for NS1 45 °C for 45 min. for reverse transcription 94 °C for 2 min for inactivation; 40 cycles of 94°C of 30 sec. 51 °C for 1 minute and 68 °C for 2 minutes. With a final extension of 68 °C for 7 min. Products were visualized with gel agarose electrophoresis at 1% and stained with ethidium bromide for 15 minutes to visualize it with a UV transilluminator.

Results and Discussion two bands one of ~408 bp and one of ~1,128 bp were amplified through RT-PCR. The first band correspond to genes prM/E and the second one to the NS1 gene confirming the presence of the WNV NS1 gene in Nuevo León, Mexico. This gene could be used as an alternative way to identify WNV in Nuevo León and even Mexico.

Conclusion Our study in base of the NS1 gene of the WNV will set the foundation for later experiments such as diagnostics tests or even vaccines. Possibly setting a new assay for future investigations regarding epitopes or even variability of new strains of the WNV reemerging as a new viral variant with a potentially epidemic outbreak.

Transposition mutagenesis in *Pseudomonas fluorescens* affects pigment producction and antagonism towards *Bacillus thuringiensis.*

Norma Elena Rojas Ruiz[1]*, Candelario Vázquez Cruz[1], Ma. Patricia Georgina Sánchez Alonso[1], Rodolfo Marsch Moreno[2].
[1] Instituto de Ciencias, Centro de Investigaciones en Ciencias Microbiológicas. Benemérita Universidad Autónoma de Puebla
[2] Departamento de Biotecnología y Bioingeniería, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Ciudad de México, México.
Benemérita Universidad Autónoma de Puebla. Edificio 103J C. U., Col San Manuel, CP. 71570, Puebla Pue. email: normaelena_rojas@yahoo.com.mx, Tel: 01222 229 55 00 Ext. 3965.

Bacillus thuringiensis has been a bacterium widely used as a biological control agent. However, its prevalence in the environment is limited by several environmental factors, such as UV light and interaction with other microorganisms present in soil, including *Pseudomonas fluorescens*. One way to characterize the antagonistic effect exerted by *P. fluorescens* on *B. thuringiensis* is to obtain affected clones in the production of toxic substances. The objetive of the present was to obtain mutants of *Pseudomonas fluorescens* by trasnposition affected in the pigments production and antagonistic effect towards *B. thuringiensis*. Two transposition reactions were carried out according to the EZ: TN^{TM} Transposon Insertion Systems of Epicentre Technologies, with a transposon that conferred resistance to spectinomycin, analyses of pigment production were carried out on solid Tris G medium with 100 µg spectinomycin. Petri dishes were incubated at 28^{o}C by 24 hours, mutant clones affected in the production of pigmentation were selected, which were tested in antagonism tests towards *Bacillus thuringiensis* in three temperatures (28, 35 and 37 ^{o}C). Spectrophotometric analyses were carried out at different wavelengths in a range from 200 to 700 nm at pH 5 and 7.5. Transposition obtained one thousand mutant clones of *P. fluorescens*, eleven of them showed to have affectation in the production of pigments with respect to the wild strain, in the tests of antagonism towards *B. thuringiensis* the results showed that the mutants have different behavior with respect to the wild strain of *P. fluorescens* in each one of temperatures employed, the spectrophotometric analyses showed that the mutants had a similar behavior in both acidic and neutral conditions, which is different from the wild type. The pioverdin pigment exhibits absorbance at wavelengths between 340 and 400 nm, for the wild strain the absorbances obtained were that agree with the reported, whereas for the transformants were lower or close to zero, therefore some mutants may be affected in the production of pioverdin.

KeyWords: *P. fluorescens, B. thuringiensis*, antagonism, mutants, transposition.

Rhizospheric microbial communities in mine tailings, a comparison of cultured and uncultured microbiomes.

Miguel Romero [1] , Diana Gallego, Jazmin Blaz, Arely Lechuga, Rocío Cruz-Ortega, Francisco Molina, Luis D. Alcaraz [1*]
[1] Laboratorio de Genómica Ambiental. Departamento de Biología Celular, Facultad de Ciencias, Universidad Nacional Autónoma de México. 04510, Ciudad Universitaria, Ciudad de México,
* lalcaraz@ciencias.unam.mx | http://genomica.fciencias.unam.mx

Dams of solid waste produced by mining operations are known as mine tailings and they are a major source of toxic contaminants such as heavy metals, which can be dispersed to nearby areas by eolic and hydric erosion. Mine tailings phytostabilization has been proposed as a bioremediation strategy to constrain the contaminants dispersion using plants to limit the effects of erosion. Rhizospheric bacteria impact plant health and facilitate plant establishment through their metabolic functions, which could be relevant in bioremediation strategies.

In this work, we have analyzed the rhizospheric communities from 4 plant species growing on mine tailings in order to explore the taxonomic and metabolic features of these microbiomes through 16S rRNA gene amplicons sequencing and the sequencing of the whole metagenome shotgun of one of these communities belonging to *Acacia farnesiana* . Besides, a combined artificial culturable community has been obtained from these rhizospheres and challenged in a synthetic ecology experiment in which microorganisms have been selected to tolerate growth in mine tailing substrate, consume plant organic compounds and compete with other members of the cultured community. The initial and final communities of this experiment were also analyzed by whole metagenome shotgun sequencing.

The taxonomic profiles of the rhizospheric communities were dominated by Proteobacteria, Actinobacteria and Acidobacteria, while the cultured fraction of these communities showed high frequency of Betaproteobacteria, Actinobacteria and Gammaproteobacteria. The resulting cultured community of the mesocosm experiment was enriched with Gammaproteobacteria, specifically by *Pseudomonas* . Comparisons between the metabolic potential of the native and cultured microbial communities showed an enrichment in genes related to amino acid and carbohydrate metabolism.

This study revealed essential taxonomic and metabolic features of rhizospheric bacteria that eventually may be exploited in bioremediation strategies to mitigate the environmental impact of mine tailings.

Identification *of Staphylococcus* in samples from patients with periodontitis by NGS.

Luis Enrique Romero Cruz, Ernesto Arturo Rojas Jiménez, Héctor Martínez Gregorio, Mario Enrique Díaz Pineda, Alejandro Rodríguez Aguilar, Luis Alberto Brito Elías, Octavio Hernández Rosas, Miriam Rodríguez Sosa, Nelly Jazmín Pacheco Cruz, Erick Alan Romero Macías, Sareni Ruiz Montoya, David Sánchez Marín, María Guadalupe Figueroa Méndez, Alina Uribe García, Claudia Fabiola Méndez Catalá, Erick Monroy Pérez, Gloria Luz Paniagua Contreras, Felipe Vaca Paniagua, Clara Estela Díaz Velásquez. Universidad Nacional Autónoma de México. FES-Iztacala. Avenida de los barrios 1, los reyes Iztacala C.P. 54090 Tlalnepantla, México. (55) 56231333 ext. 39860. cdiaz@comunidad.unam.mx

Summary

Infectious diseases caused by bacteria and yeasts are among the most frequent illnesses that affect the Mexican population. The microbiological studies of biopsies, tissues and body fluids such as urine and fecal samples, are important for an accurate diagnosis of the diseases. In the present work, bacterial strains were isolated from 12 patients with periodontal lesions at the Endoperiodontology Clinic of FES-Iztacala, UNAM. Diverse biochemical tests were used to identify bacteria, the most predominantly genus obtained in the cultures was *Staphylococcus spp*. They are one of the most common pathogens in humans, capable of causing superficial lesions on the skin, infections of the central nervous system, deep infections such as osteomyelitis and endocarditis, pneumonia and urinary tract infections. To investigate the genotypic characteristics, including resistance genes and virulence factors of these bacterial isolates, an analysis by Next Generation Sequencing (NGS) was performed. Bacterial genomic DNA was isolated and NexteraXT libraries were prepared for sequencing in MiSeq (2x300 cycles). The quality per base of the sequences was over 99% confidence (Phread Score average 37.4); average read count was 330,261, with a mean length of 138.95 bp and 34.54% of GC content (FastQC). The reads that passed the filters were used for further genomics analyses. SPades software was used for de novo genome assembly, different k-mer sizes (k21, k33, k55, k77, k99 and k127) were assessed to examine the best output (Quast software). The contigs files supplied by the assembler were used for genome annotation (Rast), species and strain determination and virulence and resistance genes identification (CGE).

A total of 5 different species of *Staphylococcus* from the bacterial isolates were distinguished: *S. epidermidis* (6/12), *S. warneri* (2/12), *S. aureus* (1/12), *S. haemoliticus* (1/12) and *S. pasteuri* (1/12). Interestingly, antibiotic resistance genes for tetracycline, *tet(K)*, and fosfomycin, *fosA*, among others were identified, as well as virulence factors for exoenzymes (*aur, spiE, spiA*) and toxins (*hlgA and hlgC*). NGS represents a powerful technique for identification and characterization of *Staphylococcus* species in periodontal lesions.

Design of a synthetic bacterial consortium to degrade polycyclic aromatic hydrocarbons

Jaime Rosas Díaz, Liliana Pardo López, Libertad Alejandra Adaya García, Karla Sofía Millán López
Universidad Nacional Autónoma de México Campus Morelos, Instituto de Biotecnología. Av. Universidad 2001, Cuernavaca, Morelos, 62210, México.
Tel.: (044) 771-204-0809 rosasdz@outlook.com

SUMMARY

Polycyclic aromatic hydrocarbons (PAHs) composed by two or more benzene rings are one of the principal components of the petroleum. Sixteen of them are classified as environmental priority pollutants according to Enviromental Protection Agency (EPA) due their toxicity, mutagenicity and carcinogenicity. Diverse genres of bacteria have been reported as main degraders of these compounds in marine environments. Our study area is the Southwest of the Gulf of México due the oil extraction processes that are carried out here. In the highly contaminated coastal region we have obtained some bacterial consortia capable of growth in presence of phenanthrene. We isolate three different bacteria from the sediment which could support growth in phenanthrene as sole carbon source. To identify the species, we analyzed their 16S gene, these isolates belong to the genders *Alcanivorax*, *Arthrobacter* and *Halomonas*. The capacity of degrading PAH was quantified after 49 days by HPLC. In this proof besides phenanthrene we also use anthracene separately. In the results obtained the best percentage of anthracene biodegradation was found in *Alcanivorax* with 20.68% ±1.11 and for phenanthrene was *Arthrobacter* with 28.55% ±13.14.

Study of the role of the proteins Avin34710 and Avin34720 in the metabolism of polyhydroxybutyrate (PHB) in the bacterium *Azotobacter vinelandii*

Jessica Ruiz Escobedo, Josefina Guzmán, Libertad Adaya, Alma Reyes González, Guadalupe Espín, Daniel Segura González.
Instituto de Biotecnología / UNAM. Av. Universidad #2001, Col. Chamilpa, Cuernavaca, Morelos, C.P. 62210,. Tel. 52 (777) 329 1629. daniel@ibt.unam.mx

Polyhydroxybutyrate (PHB) is a natural polyester synthesized by various bacteria, including *A. vinelandii*, as a reserve of carbon and energy. Bacteria accumulate PHB when there is abundance of the carbon source, and degrade it (mobilize it) when it is exhausted. This mobilization of PHB is carried out by intracellular PHB depolymerase enzymes (iPHB depolymerases), which are attached to the PHB granules, as well as the enzymes involved in its synthesis. Other PHB granule associated proteins called Phasins constitute the major protein component of the granules, although they have no catalytic activity in PHB metabolism.

In the genome of *A. vinelandii* we identified seven genes that could encode PHB depolymerases. Among them, the product of gene *Avin34710*, presents a 33% identity with well characterized PHB depolymerases.

Our analysis of the proteins associated to the PHB granules of *A. vinelandii* by mass spectrometry, revealed the presence of a new protein, which not found in any other bacterium and whose gene (*Avin34720*) is located next to the *Avin34710* PHB depolymerase gene. Because in some bacteria phasin proteins have been found to affect PHB depolymerase or PHB synthase enzymatic activities, we studied the role of the Avin34710 and Avin34720 proteins in the metabolism of PHB by generating single and double mutants inactivating these genes, and also by heterologous expression in *E. coli* for their biochemical characterization *in vitro*.

The Δ34710::Km^R mutant presented a whitish and opaque phenotype, compared to that of the wild-type strain, suggesting that the mutant could have a higher PHB content than the wild-type strain. The quantification showed that the mutation did not affect the growth and increased the specific production of PHB in *A. vinelandii* at 72 h of culture by 74%. We also characterized the enzymatic activity of these proteins expressed in *E. coli* and with these results we could verify that Avin34710 participates in the degradation of PHB and Avin34720 is involved in the synthesis of this polymer. The results suggest that Avin347109 is a PHB depolymerase that, together with the previously characterized enzyme Avin03910, participate in the degradation of PHB in this bacterium.

Regulation of the expression of microRNAs by the Zika virus during Central Nervous System development

María E. Santana Román1, Carlos Arias2, Susana López2, Victoria Pando Robles3 Gustavo Pedraza-Alva1 and Leonor Pérez-Martínez1
1Laboratorio de Neuroinmunobiología, Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología (IBt), Universidad Nacional Autónoma de México (UNAM), Cuernavaca, Morelos 62210, México. 2Departamento de Genética del Desarrollo y Fisiología Molecular, IBt-UNAM, Cuernavaca, Morelos 62210, México. 3Instituto Nacional de Salud Pública, Universidad No. 655 Colonia Santa María Ahuacatitlán, Cerrada Los Pinos y Caminera C.P. 62100, Cuernavaca, Morelos. México. Correspondence: leonor@ibt.unam.mx

The Central Nervous System (CNS) is composed of two cell types: neurons and glial cells. These cells, derived from the neuroectoderm, come from neural stem cells (NSC)/neural progenitors (NPs) characterized by a broad self-renewal potential. NSCs and NPS are encountered in both embryonic and adult brain. NPs divide asymmetrically and one of the two daughter cells irreversible exits of the cell cycle giving rise to a neuron. For neurogenesis to occur, cellular processes such as proliferation and gene expression regulation must be tightly regulated. In recent years, it has been shown that gene expression during CNS development can be regulated at post-transcriptional level by small non-coding RNAs specifically, microRNAs (miRNAs). Due to the high expression of miRNAs in the developing brain, it has been suggested they are important elements in gene expression regulation, controlling proliferation, cell differentiation and inhibition of apoptosis. Interestingly, the miRNAs can be regulated by inflammatory signals such as cytokines (e.g. TNF and interferon (IFN). Recently, it has been an important increase worldwide in microcephaly cases caused by infections with the Zika virus (ZIKV). This RNA+ virus infects cells located in the ventricular and subventricular zone of the developing cortex, causing increase in apoptosis, reduction in the NPs mitotic potential and inhibition of cell differentiation. In addition, ZIKV infection increases the expression of cytokines (e.g. TNF, IFN), toll-like receptors (TLR 3/4 and 7) and activate the transcription factors IRF3, IRF7 and, NF-kB. Therefore, here we propose that ZIKV induces microcephaly via upregulating the expression specific miRNAs within the CNS.

The miRNAs regulated by the ZIKV (Asian and African lineage) in the N1 cell line will be presented. Current experiments are aimed to determine the proliferative (BrdU, Ki67) and differentiation capacity (Neu, MAP2, GFAP) of the N1 infected cells. A model of Zika virus infection in vivo will be established and the brain development of the progeny will be determined.

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Keywords: microcephaly, Zika virus, neuroinflammation, neurogenesis, cell differentiation.

Paper of CsrA in the growth of *Bacillus licheniformis* M2-7 in the presence of hydrocarbons

Laura Iztacihuatl Serrano Ángel, Daniel Segura Gonzáles, Jeiry Toribio Jiménez, and Yanet Romero Ramírez.
Laboratorio de Microbiología Molecular y Biotecnología Ambiental, Universidad Autónoma de Guerrero, México. Av. Lázaro Cárdenas s/n. Ciudad Universitaria. Apdo. Postal 39070, Chilpancingo, Guerrero.
Instituto de Biotecnología, Universidad Nacional Autónoma de México. Departamento de Microbiología Molecular. Avenida Universidad No. 2001. Colonia Chamilpa. Cuernavaca, Morelos, CP 62210
yanetromero7@gmail.com; itzylura_80@hotmail.com

The pollution for hydrocarbons in the environment represents 70% of the total ecosystems impacted (Ortiz, 2005). The presence of hydrocarbon degrading bacteria is one of the main mechanisms used by certain environments to mitigate the impact caused by the presence of hydrocarbons and their derivatives. These microorganisms, when in contact, consume them as the sole carbon source (Leahy and Colwell, 1990, Head et al., 2006). This through the development of various mechanisms developed to detect and respond to changes, one of them being regulation at the post-transcriptional level, where the Carbon Storage Regulator (Csr) or Repression of secondary metabolites (Rsm) (Babitzke and Romeo).
The global system Csr in bacteria is a regulator in the exponential and stationary phase of growth, is conserved in different bacteria and is responsible for controlling the gene expression of many important cellular functions such as carbon storage, mobility, pathogenicity, quorum sensing, virulence and biofilm formation (Romeo, 1998; Esquerré et al., 2016; Figueroa et al., 2014; Broberg et al., 2014).
This system consists of two elements, CsrA, a messenger RNA binding protein, and CsrB, a non-translatable RNA (sRNAs) (Suzuki et al., 2002; Vakulskas et al., 2016). The mRNA-binding protein CsrA is a key regulator of a variety of cellular processes in bacteria, including carbon metabolism in the stationary phase (Tesfalem et al., 2015). CsrA act as a translational repressor (Babitzke and Romeo, 2007); typically CsrA binds to multiple conserved sequences (5'UACAGGAUGU'3) in the white transcripts, in most cases at least one binding site overlaps with the Shine-Dalgarno (SD) sequence. Therefore, CsrA inhibits translation by blocking access of the ribosome to mRNA (Romeo, 1998, Wei et al, 2001, Vakulskas et al, 2015). The functions regulated by this system of global regulation Csr or Rsm has been discovered and studied mostly in various Gram negative bacteria ranging from central carbon metabolism, biofilm, virulence and motility in *E.coli* (Suzuki et al. , 2002; Tomenius et al., 2006), in *Campylobacter jejuni*, regulates resistance to oxidative stress and the formation of biofilm (Fields and Thompson, 2008). However, in Gram-positive bacteria only studies in *Bacillus subtilis* have been reported.
Within the genus Bacillus, the *licheniformis* and *subtilis* species share a very similar physiology, as well as the mechanisms of cellular transport and protein production (Ageitos, 2012).
Research carried out in the Laboratory of Molecular Microbiology and Environmental Biotechnology has shown that the strain *B. licheniformis* M2-7 is capable of degrading petroleum hydrocarbons, gasoline, diesel and burnt oil. This was proven by cultivation in Bushnell-Haas (BH) medium with these hydrocarbons as the sole carbon source (unpublished data). It was also observed that it is able to grow in the presence of four Polycyclic Aromatic Hydrocarbons (PAHs): naphthalene, phenanthrene, pyrene and benzo [a] pyrene and it was shown that it can biotransform benzo [a] pyrene to tonic acid (Guevara et al., 2018). In order to begin to decipher the regulatory networks that would be activating the hydrocarbon metabolism in our study model, we searched for the csrA gene in the genome of *Bacillus licheniformis* M2-7 and it was amplified by PCR. However, the function of this system is not known in the diverse cellular processes of said bacterium, among them, the degradation and growth on hydrocarbons. Therefore, the objective of this work is to evaluate if the CsrA protein regulates the use of hydrocarbons in *Bacillus licheniformis* M2-7. This is important for the search for thermo-resistant bacteria with a greater capacity to degrade hydrocarbons, because in the state of Guerrero and nationally there are no effective bioremediation strategies on the sites where the hydrocarbons are used.

Do the SPFH-containing proteins affect secretion in *Escherichia coli*?

Lidia Steinemann Hernández, José Enrique Guzmán Flores, Elizabeth García Gómez, Claudia Rodríguez Rangel, Norma Espinosa, Adrián Álvarez, Bertha González Pedrajo, Dimitris Georgellis.
Instituto de Fisiología Celular

Departamento de Genética Molecular, Instituto de Fisiología Celular, Circuito Exterior s/n Ciudad Universitaria, Coyoacán, México D.F., C.P. 04510; Tel: 56225738; steinemann@email.ifc.unam.mx

Membrane lipid rafts have been broadly studied on eukaryotic cells. These membrane structures have been associated with diverse cellular processes such as signal transduction and protein transport. Interestingly, a set of proteins, all of which have the SPFH domain (Stomatin, Prohibitin, Flotillin, HflK/C), is always associated with the eukaryotic lipid rafts. Accordingly, disruptions of lipid rafts or SPFH-protein mutants are associated with a large variety of diseases. Nevertheless, it was not until recently that such membrane microdomains were reported to exist in bacteria, and a method for their isolation from E. coli inner membranes was reported.

Here, we present our results aiming at elucidating whether mutants of the SPFH-containing proteins affect the assembly and function of the E. coli secretion systems.

Point mutation of the hot-spot E176 in the capsid protein of the Cowpea Chlorotic Mottle Virus decreases its thermal stability

Alejandra G. Valdez-Lara, Mariana Andrade-Medina, and Mauricio Carrillo-Tripp*
Biomolecular Diversity Laboratory, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional Unidad Monterrey, Vía del Conocimiento 201, Parque PIIT, C.P. 66600, Apodaca, Nuevo León, México
E-mail: mauricio.carrillo@cinvestav.mx

Abstract

Viruses are the most abundant pathogens affecting all forms of life. A major component of a virus is a protein shell that encapsulates the genomic material known as the viral capsid. The capsid has the fundamental functions to protect and transport the viral genome and to recognize the host cell. Specific protein-protein interactions direct the assembly of the capsid. Nevertheless, our understanding of the molecular mechanisms involved in such a process is limited. Based on structural conservation, we have proposed the existence of hot-spots, a small set of residues in the capsid‘s inter-subunit interfaces that significantly contribute to the free energy of formation. We have previously identified the hot-spots of the Bromoviridae family, non-enveloped icosahedral phytopathogenic viruses with enormous impact and applications on the agriculture industry of Mexico.

Here, we experimentally characterize the thermodynamic effect on the capsid‘s assembly and stability by the single mutation of hot-spots in the Cowpea Chlorotic Mottle Virus, a member of the Bromoviridae family. Thermal shift assay stability curves show that when a single hot-spot is mutated, e.g., E176, the melting point of the whole capsid decreases significantly. A randomly selected interface residue does not alter the capsid‘s melting point when mutated. These results support the idea that hot-spots are responsible for the capsid‘s stability and shed new light on the molecular mechanism of capsid assembly.

Handrail and turnstiles microbiome of the Mexico City Subway

Daniela Vargas-Robles, Apolinar Misael Hernández, Luis D. Alcaraz, Mariana Peimbert*

Universidad Autónoma de Metropolitana, Unidad Cuajimalpa (UAM-C)
Avenida Vasco de Quiroga 4871, Santa Fe Cuajimalpa, 05370 Cuajimalpa, CDMX.
(55) 5814 6500 ext 3806, marianapeimbert@gmail.com

Bacteria in urban environments are associated to human microbiome, plants, pets, ventilation systems and architectural designs. The subway is one of the more representative urban systems of Mexico City. It is one of world largest transport system and moves around 4.5 million people daily. This big and constant congregation of people promotes the exchange of human and environmental microbes. In this study we determined the bacterial diversity and composition of the Mexico City subway.

Samples from turnstiles (N=24) and vertical handrails inside de train (N=23) were taken swabbing their surfaces. DNA was extracted from samples, 16S rRNA gene was amplified and sequenced with Illumina MiSeq. Sequences were processed and analyzed.

We obtained close to 5 million sequences assign to 50,197 Operational Taxonomic Units (OTUs, no singletons). The most representative genera were *Propionibacterium* spp. (15%; *P. acnes* 13%), *Corynebacterium* spp. (13%), *Streptococcus* spp. (9%) and *Staphylococcus* spp. (5%; *S. epidermidis,* 4%), all associated with the human body, other genera were present in less than 4% of relative abundance. Bacterial source was mostly from skin and dust, followed by saliva and soil. Composition and diversity of bacteria was independent of humidity, temperature, train depth or train line. However, bacterial composition of turnstiles and handrail were significantly different and turnstiles showed higher diversity than handrail. Additionally, although salivary bacterial contribution was similar for both sites, dust and soil were higher in turnstile and skin higher in handrails.

This study showed that microbial composition of the Mexico City subway comes from a mixture of environmental and human sources and that users are exposed to normal human flora but also to opportunistic bacteria that may be a relevant for hosts with weakened immune systems.

Overproduction of rhamnolipids in *Pseudomonas aeruginosa* ATCC 9027.

Paola Vázquez-Bueno, Abigail González-Valdez, Uriel Gutiérrez-Gómez, Gabriel Ponce-Soto, Miguel Cocotl-Yañez, Gloria Soberón-Chávez.
Department of Molecular Biology and Biotechnology, Institute of Biomedical Research, UNAM. P.O.B. 70228, 04510. gloria@biomedicas.unam.mx

Pseudomonas aeruginosa is an environmental bacterium that produces compounds of biotechnological importance such as the biosurfactants rhamnolipids (RHLs). The majority of RHLs produced by this bacterium correspond to: mono-rhamnolipids (α-$_L$-rhamnopyranosyl-β-hydroxydecanoyl-β-hydroxydecanoate) and diramnolipids (α-$_L$-rhamnopyranosyl- α-$_L$-rhamnopyranosil-β-hydroxydecanoyl-β-hydroxydecanoate). RHLs biosynthetic pathway begins with the uptake of fatty acids by RhlA that produces a fatty-acid dimer, that is one of the substrates of RhlB rhamnosyl-transferase, which catalyzes the formation of mono-RHL using this fatty acid-dimer and TDP-$_L$-rhamnose as the second. In turn, RhlC rhamnosyl-transferase uses mono-RHL and TDP-$_L$-rhamnose as substrates and produces di-RHL. The high-scale production of these compounds is limited by the potential virulence of *P. aeruginosa* and by the high production costs. As an alternative option to the large scale production of RHLs, non-pathogenic *P. aeruginosa* strains or heterologous strains have been designed with little success.

RHLs production is regulated at the level of transcription by the quorum-sensing response (QSR), particularly the transcription of the *rhlA* and *rhlB* genes depend directly on the RhlR transcriptional regulator coupled with the autoinducer butanoyl homoserine lactone (C4-HSL).

In this work we present ATCC 9027 *P. aeruginosa* strain as a model for the overproduction of RHLs, since it has been shown to be avirulent in a mouse model. Its inability to infect has been associated with the lack of SST3, due to a deletion of 20 kb. Regarding RHL production it has been described that it only forms mono-RHL due to a natural *rhlC* deletion.

Our studies have been conducted in low phosphate medium (PPGAS) that has been shown to favor the production of RHLs. We elaborated kinetics of growth and production of RHLs, concluding that the highest production occurs in stationary phase at 24 hours of culture.

In addition, we expressed in trans the *rhlC* gene of the *P. aeruginosa* type strain PAO1 to increase total RHLs production and see if we could obtained only di-RHLs. The highest total RHLs production (consisting of both mono- and di-RHLs) was obtained when *rhlC* was expressed constitutively from plasmid pUCP24, compared to the expression of a vector that expressed this gene from a plasmid with a promoter induced by arabinose addition. The production of mono-RHL by strain ATCC 9027/pUCP24-*rhlC* can be mainly due to: a) the limitation of the concentration of TDP-$_L$-rhamnos, or b) the production of mono-RHL by RhlC as a by-product of the reaction of di-RHLs synthesis. It has been previously shown that mono-RHLs production was increased in strain ATCC 9027 by the overexpression of *rhlABR, so* we decided to construct vectors that express *rhlC* together with *rhlR* or with *rhlABR* to further increase the production of these biosurfactants. These experiments are in progress.

ZMP dependent activation of response regulators in *Escherichia coli*

Oscar Jair Vázquez-Ciros, Alejandro Madrigal Carrillo, Alfredo Torres-Larios, Adrián Fernando Alvarez and Dimitris Georgellis
Instituto de Fisiología Celular
Departamento de Genética Molecular, Instituto de Fisiología Celular, Circuito Exterior s/n Ciudad Universitaria, Coyoacán, México D.F., C.P. 04510; Tel: (+52) 5556225738;
ovazquez@email.ifc.unam.mx

Abstract
Bacterial two component signal transduction systems (TCS) are molecular circuits that allow microorganisms to detect, amplify and respond to diverse stimuli. A typical TCS is comprised by a membrane bound histidine kinase protein (HK) and a cytosolic response regulator protein (RR). Signal perception by the HK stimulates an ATP-dependent autophosphorylation at a conserved histidine residue, which then donates the phosphoryl group to an aspartate residue in the cognate RR. In the absence of the cognate HK, RRs have been shown to autophosphorylate at the expense of the high-energy phosphate compounds acetyl phosphate and carbamoyl phosphate.
In this study we present experiments demonstrating that ZMP (5-amino-4-imidazole carboxamide riboside-5'-monoposphate), an intermediate of the purine synthesis, can also induce the activation of RRs in a HK-independent manner

Effect of CsrA of *Bacillus licheniformis* on mobility and ability to grow on various carbon sources

Isabel Villa Morales, Juanita Jacobo Valerio, Laura I. Serrano Ángel, Miguel Á. Rodríguez Barrera, Jeiry Toribio Jiménez, Verónica Antonio Vejar y Yanet Romero Ramírez
Laboratorio de Microbiología Molecular y Biotecnología Ambiental, Universidad Autónoma de Guerrero, México. C.p. 39070. Cel: 7471020121. Correo: yanetromero7@gmail.com

The carbon storage regulator, or Csr, is a global regulation system that controls bacterial gene expression post-transcriptionally[1]. This system controls a wide variety of physiological adaptive mechanisms such as virulence, quorum sensing, cell motility, biofilm development and carbon metabolism; it is constituted depending on the bacterial species by two to four fundamental molecular components[2]. The main component is the post-transcriptional regulator CsrA, a protein that it influences both translation and degradation of different mRNA targets[3]. In the laboratory, the *Bacillus licheniformis* strain M2-7 is studied as a study model. This strain was isolated from hot springs of the Coacoyul community, municipality of San Marcos, Guerrero.It is a heat-resistant bacterium since it survives at a temperature of 60 °C and can resist up to 110 °C, because it has proteins that are thermostable. A strain derived from *Bacillus licheniformis* M2-7 was constructed, which is blocked in the csrA gene by means of a cassette of spectinomycin resistance. In this project this strain of modified *Bacillus licheniformis* was characterized on mobility and the ability to grow under different sources of carbon, in order to determine the influence of this gene on the phenotypes raised, mobility tests were carried out in LB medium at 0.9% agar. As results, we obtained that the strains blocked in the csrA gene have greater mobility than the wild strain. This is related to the phenotypes reported for strains blocked in csrA in other organisms.

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Preservation of the erythrocyte band 7 integral membrane protein to damage of serine proteases (SPATE) from enteroaggregative *Escherichia coli.*

Jorge Mateo Villaseca Flores[1], Cecilia Martínez Castillo[1], Alan Elías Almonte Dorantes[1], Sergio Iván Jasso Campuzano[1], Andrés Reyes Valdés[1], Yolanda Medina Flores[2], Olga Mata Ruiz[2]. [1], Laboratorio de Bacterias Patógenas, Departamento de Microbiología y Parasitología. Facultad de Medicina, UNAM. Av. Universidad 3000, Col. UNAM, CU, Del. Coyoacán, C. P. 04510, CDMX., Tel.: 56232501, Correo-e: villasec@unam.mx. [2], Laboratorio de Anticuerpos Monoclonales, Departamento de Investigaciones Inmunológicas, INDRE, SS. Francisco de P. Miranda 177, Lomas de Plateros, 01480, CDMX.

Enteroaggregative *Escherichia coli* (EAEC) is a commonly cause of endemic and epidemic diarrhoea in both developed and developing countries. EAEC colonization can occur in the mucosa of both the small and large bowels, which can lead to mild inflammation in the colon. Diarrhoea caused by EAEC is watery and it can be accompanied by mucus or blood. EAEC produce the toxins Pic (Protein involved in intestinal colonization) and Pet (Plasmid-encoded toxin), both proteins are members of the Serine Protease Autotransporters of the *Enterobacteriaceae* (SPATE) family from *Escherichia coli* and *Shigella* spp., which possess a consensus serine protease motif (GDSGS^{260}GV). Functional analysis of Pic implicates this factor in mucinase activity, serum resistance, and hemagglutination. Pet is capable of cleaving spectrin of erythrocyte ghosts, purified spectrin, and fodrin (nonerythroid spectrin) from HEp-2 cells. The erythrocyte membrane protein band 7 (stomatin) is a 31 kDa integral membrane protein that regulate ion channels and transporters. Also, this protein plays a structural role in the membrane and anchorage of the actin cytoskeleton.
Band 7 is a probable target of the SPATEs. The objective of this study was to analyzethe damage of SPATE from EAEC on the erythrocyte band 7 integral membrane protein. The SPATEs (Pic and Pet) were purified from the Luria broth culture of the EAEC 049766 strain, producing both toxins. Proteins were obtained by ammonium sulphate precipitation and the proteins were fractionated with potassium buffer. The biological activity of STATEs on erythrocyte membrane proteins was assay. The protein fractions were incubated with horse erythrocytes suspension for different times. They were washed with saline solution and an osmotic shock was produced with hypotonic phosphate solution. Finally, the ghosts of erythrocyte were washed with saline solution. The membranes were analyzed by SDS-PAGE in reducing conditions. The degradation of 240 and 220 kDa protein corresponding to alpha and beta spectrins was observed. The low molecular weight protein of 31 kDa not showed proteolysis. This effect of preservation was higher in the protein fraction that preferably contains the SPATEs. Which indicates that the SPATEs not damage the band 7 to the cell membrane. The preservation of the band 7 integral membrane protein to damage of serine proteases (SPATE) from EAEC can be a process of compensation for the exchange of ions through the cell membrane, where was alter the organization of the cytoskeleton.

Características histopatológicas y detección de Papilomavirus Bovino en la Fibropapilomatosis Bovina en la región centro norte de México

Histopatological characteristics and Bovine Papilomavirus detection in bovine fibropapylomatosis in the northern México region

Isaura Méndez Rodríguez, [1] Fernando Alberto Muñoz Tenería, [1] Milagros González Hernández, [1] Alan Martínez Castellanos, [1] Luisa Eugenia del Socorro Hernández Arteaga[1*]
[1] Facultad de Agronomía y Veterinaria, Universidad Autónoma de San Luís Potosí. Carretera San Luis-Matehuala Km. 14.5, Ejido Palma de la Cruz, C.P. 78321 Soledad de Graciano Sánchez, SLP, México
*Correo electrónico: LESHA: socorro.hernandez@uaslp.com

Abstract

Bovine fibropapillomatosis is a specific viral disease, caused by bovine papillomavirus (BPV) which is able to induce the formation of benign neoplasms in the form of papillomas, fibropapillomas and / or predispose to the development of malignancies such as squamous cell carcinoma in different organs of the animal. The aim of this work was to determine the presence of BPV in tissue samples of cattle with suggestive skin lesions of papillomas, fibroids and fibropapillomas in production units of cattle of different regions of the state of San Luis Potosí. We obtained incisional and excisional biopsies of bovine skin with suggestive lesions of papillomas, fibropapillomas and squamous cell carcinomas. Samples were processed by histopathology and DNA was extracted for BPV detection by PCR with the Oligonucleotides FAP59 / FAP64 and MY09 / MY11.

Based on histopathological characteristics of the tissues, these were classified as fibromas (45.45%) and fibropapillomas (54.54%) without distribution of a specific type of lesion according to anatomical location, age or production system.

72.72% (n = 8) of the samples tested showed positive results for the detection of BPV by PCR; 45.45% (n = 5) of the positive samples with the FAP oligos and 54.54% (n = 6) with the MY oligo set. We did not found a statistically significant association between presence of BPV and the breed of cattle, age or production system. On samples that showed negative results to the amplification by PCR (27.27%), histopathological lesions were strongly suggestive of a viral infection, which is why we can‘t definitively rule out association of these with some type of BPV. As far as we know, this is the first study that describes the prevalence of PVB in animals of the state of San Luis Potosí, so these results provide useful data to establish detection and control measures necessary to improve animal health conditions.

Key words: Bovine papillomavirus, Histopathology, PCR, San Luis Potosí

Fc gamma RIIIb induces extracellular calcium influx without mobilization of calcium from intracellular deposits, in human neutrophils.

Omar Rafael Alemán, Nancy Mora, and Carlos Rosales.
Instituto de Investigaciones Biomédicas. UNAM.
Circuito Mario de la Cueva s/n, Coyoacán, Ciudad Universitaria, 04510 Ciudad de México. Tel: 56228951. carosal@unam.mx.

Polymorphonuclear leukocytes (PMN) or neutrophils are the first cell type to arrive at the infected site for eliminating pathogenic microorganisms. PMN are able to recognize opsonized pathogens via receptors for antibodies (IgG), known as Fc gamma receptors (FcγRs). The human PMN express the receptors FcγRIIa and FcγRIIIb. Previously, we have shown that FcγRs mediate different functions. FcγRIIa induces phagocytosis, while FcγRIIIb induces formation of neutrophil extracellular traps (NETs). Also, these two FcγRs can induce calcium (Ca^{2+}) mobilization. FcγRIIa uses IP_3 to release Ca^{2+} from intracellular deposits and provokes an increase in cytosolic Ca^{2+} concentration. FcγRIIIb does not use IP_3 for Ca^{2+} mobilization, suggesting that FcγRIIIb may not release calcium from intracellular deposits. In order to determine whether FcγRIIIb is able to induce calcium mobilization from intracellular deposits, neutrophils were loaded with Fura-2 and cytosolic Ca^{2+} concentration was determined in calcium free conditions after FcγR stimulation. FcγRIIa, as previously reported, was able to induce both intracellular and extracellular calcium mobilization. In contrast, FcγRIIIb did not induce intracellular calcium mobilization from internal deposits, but was able to induce calcium mobilization from outside the cell. Our findings suggest that calcium channels on the membrane of the neutrophil are activated by FcγRIIIb to provoke an increase in cytosolic Ca^{2+} concentration.

Role of p53 and mutants (R175H and R273H) over canonical pathway of Wnt on a model of Cancer Stem Cells.

Eduardo Alvarado Ortiz[1,2], Elizabeth Ortiz Sanchez[2], Alejandro García Carrancá[1,2]

1 Laboratorio de Virus y Cáncer, Unidad de Investigación Biomédica en Cáncer, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Av. San Fernando #22, Colonia Sección XVI, Tlalpan, CP 14080, México.

2 División de Investigación Básica, Instituto Nacional de Cancerología, Secretaría de Salud, México. Av. San Fernando #22, Colonia Sección XVI, Tlalpan, CP 14080, Mexico. E-mail carranca@biomedicas.unam.mx

Cancer Stem Cells (CSC) constitute a minor subpopulation able to self-renew and differentiate to rest of cells that constitute the tumoral mass. One of the major elements involved in the self-renewal process is the Wnt pathway. The canonical mechanism consist on regulating the levels and stability of β-Catenin, which functions as a transcriptional co-activator that contributes to the generation and maintenance of CSC. The p53 protein is a tumor suppressor that is commonly mutated on different types of cancer, and has been show to exert a suppressor activity on the Wnt pathway. Taking as starting point this premise, the present work propose to study the participation of Wt-p53, as well as of mutants R175H and R273H on enriched cultures of CSC and its effect of Wnt pathway regulation. Employing as model of study H1299 cell line (null to p53), overexpression assays of Wt-p53/R175H-p53/R273H-p53 were done on monolayer cultures, and exhibited a significant decrease of proliferation capacity by Wt-p53. The effect of Wt-p53 overexpression generate an increase of p21, a direct target of functional version of p53 and decreases the presence of proteins associated to Wnt pathway, including β-Catenin and LRP, a co-receptor of Wnt ligand. This effect, observed at 72 hrs post-transfection, is not observed by mutants. Additionally, the overexpression of Wt-p53, but not mutants (R175H/R273H) decreases the capacity of this cell line to generate spheroids on enrichment cultures of CSC, the total levels of β-Catenin not have changes, but interestingly the effect of mutants suggest differential mechanisms over p21 that highlights the importance of the study and repercussion of p53 mutants, likewise support the role of negative regulation generated by p53 on Wnt pathway, intimately related to CSC maintenance.

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Mechanisms of secretion of the TGF-β cytokine from melanoma cells and its actions on mast cells

Isabel Anaya Rubio1, Alfredo Ibarra-Sánchez2, Claudia González Espinosa2, and Marina Macías Silva1
1Departamento de Biología Celular y Desarrollo, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Ciudad de México. Tel. +52 55 56-22-5729. 2 Departamento de Farmacobiología, Cinvestav-IPN. Ciudad de México. ianaya@email.ifc.unam.mx

Abstract
Melanoma is a type of neoplasm that results from the malignant transformation of melanocytes, being the most lethal and aggressive of cutaneous neoplasias. One of the most important risk factors for the development of this type of cancer is intense exposure to ultraviolet radiation, but there are other factors such as skin type, family history of melanoma, genetic susceptibility, environmental factors and immunosuppression. Genetic alterations in melanoma occur in different oncogenes including: NRAS, BRAF, KIT, and MEK1 (MAPK2K1), as well as in tumor suppressors such as p53 and CDKN2A ($p16_{Ink4A}$); there is also an alteration within the proto-oncogenes that belong to the growth factors and their receptors, as in the case of Transforming Growth Factor beta (TGF-β). Melanoma cells have high levels of the TGF-β cytokine. TGF-β is a pleiotropic cytokine that is involved in the development of different neoplasms. In normal cells, TGF-β acts as a tumor suppressor, inhibiting cell growth and differentiation. On the other hand, in malignant cells, it acts as a pro-tumorigenic agent, activating different processes, such as autocrine or paracrine pathways that promote cell proliferation, tumor angiogenesis, epithelial- mesenchymal transition of tumor cells, migration, evasion of the immune system and metastasis. It is known that this cytokine controls the initiation and termination of inflammatory responses, by regulating chemotactic processes in different cells, including the mast cells. The mast cells are cells of the innate immune system, which under normal conditions participate in the regulation of inflammatory response and allergic reactions. They also play an important role in the development of neoplasms by promoting angiogenesis and secreting mediators that stimulate endothelial cells to form new blood vessels. Based on this background, this project aims to evaluate the mechanisms of secretion of the TGF-β cytokine by melanoma cells, as well as studying the actions of this cytokine as a chemoattractant of the mast cells for the progression of melanoma. So far, we have found that TGF-β is expressed in exosomes derived from melanoma cells, and that this route of secretion is possibly important for the chemotaxis of cells of the immune system, such as mast cells. We believe that the knowledge generated will allow to understand part of the behavior and the development of this type of neoplasias.

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Heterologous calcium-dependent inactivation of Orai by neighboring TRPV1 channels modulates cell migration and wound healing

Carlos Ernesto Bastian-Eugenio, Arlette Bohorquez, Alexander Asanov, Jonathan Pacheco, Jose Ocelotl-Oviedo4, Adán Guerrero, Alberto Darszon and Luis Vaca*
*Corresponding author: Dr. Luis Vaca, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Ciudad Universitaria. Mexico CDMX 04510. Tel. (52)55 56225654. Email: lvaca@ifc.unam.mx

Calcium ion (Ca^{2+}) is a second messenger important in numerous cellular processes. Store operated calcium entry (SOCE) is the main mechanism for calcium mobilization in non-excitable cells. The essential components of SOCE are the endoplasmic reticulum (ER) Ca^{2+} sensor STIM1 and the plasma membrane (PM) channel Orai. Orai activity is regulated through a negative feedback mechanism that maintains intracellular Ca^{2+} homeostasis and prevents excessive Ca^{2+} influx. Such mechanism is known as Ca^{2+}-dependent inactivation (CDI). CDI consists of slow CDI (SCDI) and fast CDI (FCDI), which have distinct kinetics and sites of action. FCDI take place within ~10 to 100 milliseconds after channel activation and is controlled by Ca^{2+} binding to a site located ~8 nanometers from the channel pore.
All the studies carried out to this date to explore CDI in Orai1 have been conducted by artificially increasing intracellular Ca^{2+} via the patch clamp pipette, which reflects CDI induced by Ca^{2+} entering through the Orai channel pore (homologous CDI). Less studied are physiological sources of Ca^{2+}, such as other channels. In the present study we have explored other sources of Ca^{2+} arising from different channels. We have found that Ca^{2+} entering the cell trough TRPV1 channels induce strong CDI in Orai1, while Ca^{2+} entering trough P2X4 purinergic channels does not. Super resolution, co-immunoprecipitation and Förster resonance energy transfer (FRET) studies indicate that Orai1 and TRPV1 are associated and move in close proximity to each other at the PM, while P2X4 and Orai1 do not. These results indicate that a close association between TRPV1 and Orai1 result in an elevated Ca^{2+} microenvironment when TRPV1 channels are activated, which enhances CDI in Orai1. Because P2X4 and Orai1 are not found in proximity, Ca^{2+} entering P2X4 channels do not affect CDI in Orai1, in spite the fact that Ca^{2+} entering trough P2X4 contribute to increments in cytosolic Ca^{2+} concentrations. Deletion analysis and peptide arrays show that the first ankyrin domain (ANK1) in TRPV1 is required for the association of this channel to the N-terminal domain from Orai1. This ANK1 domain maintains both channels close and favors CDI in Orai1. Deleting this domain reduces significantly CDI in Orai1 elicited by Ca^{2+} entering trough the TRPV1.
Our results have important physiological implications in the modulation of Ca^{2+} influx in cells where TRPV1 and Orai1 channels coexist, such as astrocytes. We show that TRPV1 is an important modulator of Orai1 channel activity in cortical astrocytes by controlling CDI in this channel and thus reducing the amount of Ca^{2+} entering to the cell when TRPV1 and Orai1 are simultaneously or sequentially activated. This heterologous modulation of CDI plays a role in controlling cell migration and wound healing in astrocytes.

Evaluation of anticancer capacity of exotic fruits: *Nephelium Lappaceum, Melicoccus bijugatus, Manilkara zapota* via the modulation of intracellular SUMOylation processes

Gisela M Basto-López., Oscar Ruiz Castellanos, Ricardo Solano Ascencio, Elena M Cuevas Sánchez, Vanessa Dehennaut †, José A González Garrido, Carlos J Victorio López, Oswaldo I Hernández Abreu, Edgar Zenteno Galindo ‡, Adelma Escobar-Ramírez *
*Juárez Autonomous University of Tabasco, academic division of basic Sciences, Carretera Cunduacán-Jalpa Km1. Col. La Esmeralda, CP 86690, Cunduacán, Tabasco, México, adelmaescobar@yahoo.com.mx, 914 336 09 28 ext.6702.
† Institute of biology of Lille, Mechanisms of Tumorigenesis and Targeted Therapies UMR 8161 CNRS, 1 rue du Pr Calmette CS50447, 59021 Lille Cedex, France
‡ Biochemistry department of UNAM, Av. Universidad 3000, Cd Universitaria, 04510 Coyoacán, CDMX.

Cancer, the second leading cause of death worldwide, caused 8.8 million deaths in 2015. Nearly one in six deaths worldwide is due to cancer. About 70% of cancer deaths occur in low- and middle-income countries. About one third of cancer deaths are due to the five main behavioral and dietary risk factors: high body mass index, lack of physical exercise, smoking, alcohol, and low fruit and vegetable consumption. On this last point, it is well known that exotic fruits have important quantities of phenolics acid, flavonoids, tannins and others compounds that possesses antioxidant and/or anticancer properties. In this work, we evaluated the antioxidant/anticancer properties of different extracts from *Melicoccus bijugatus, Nephelium lappaceum, and Manilkara zapota,exotic fruits* endemic in the south of México. For that, the skin, pulp and seeds of each fruit were collected separately. The seeds have been previously dried. The skin and pulp were macerated and extracted with methanol using phytochemical techniques. After removal of the solvent with a rotary evaporator, the extract obtained was lyophilized, and its cytotoxicity was measured on three cancer cell lines: colic (HCT116), prostatic (DU145) and breast (MCF7) by Incucyte® cell proliferation assays and MTT cell viability assays. *Manilkara zapota* seeds show the best antiproliferative properties. In a second part of this work, we investigated the cellular mechanisms by which these compounds could affect cancer cells proliferation and viability. We focused on SUMOYlation processes, a post-translational modification of proteins consisting in the covalent linkage of one or several residues of SUMO-1 or SUMO-2 (Small Ubiquitin Like Modifier) onto lysines of the targeted proteins that control different cell processes like transcription, genome integrity, nuclear trafficking and cellular signalization and whom dysregulation has been implicated in cancer development. We hypothesized that phenolic acids could modulate cancer cells SUMOYlation levels. To test this hypothesis, we evaluated the effect of our different fruit extracts on SUMO-1 and SUMO-2 levels by Western Blot using specific antibodies. Our preliminary results show that total extracts containing phenolic acid impact the sumoylation level in cancer cell lines. *Manilkara zapota* seeds extract, showed a low level in SUMO 2/3 and SUMO1 on HCT116 cell lines.

P-Rex1, a signaling platform and effector of the Gβγ-PI3Kγ-mTORC1/2 pathway involved in cell migration

Yarely Mabell Beltrán-Navarro1, Sendi Rafael Adame-García2, Rodolfo Daniel Cervantes-Villagrana1, Guadalupe Reyes-Cruz2 and José Vázquez-Prado1.
Departments of 1Pharmacology and 2Cell Biology. Cinvestav-IPN.
Av. IPN 2508, Col. San Pedro Zacatenco, C.P. 07360 CDMX.
Tel: 5747 3800
E-mail: jvazquez@cinvestav.mx

P-Rex1 integrates chemotactic signals transduced by CXCR4 and other G-protein coupled receptors (GPCRs) via heterotrimeric Gi that releases Gβγ activating PI3K, among other effectors. P-Rex1, a guanine nucleotide exchange factor (GEF) for the Rac GTPase, can be independently and synergistically activated by Gβγ and phosphatidylinositol-3,4,5-trisphosphate (PIP3), a lipidic second messenger produced by class I PI3K. By modifying the actin cytoskeleton, this pathway promotes polarized cell migration. Gβγ seems to directly stimulate P-Rex1-DH domain and PIP3 interacts with P-Rex1-PH domain. Our group has demonstrated that mTOR, a multifunctional kinase organized in two independent complexes, interacts with P-Rex1-DEP domains. Additional unpublished data revealed potential interactions with PI3K and additional Gβγ-interaction regions. Therefore, we postulate that P-Rex1 serves as a platform and signaling effector of the Gβγ-PI3Kγ-mTORC1/2 pathway involved in chemotactic cell migration.
We assessed this hypothesis by isolating the active fraction of P-Rex1 from SDF1-stimulated cells, exploring the presence of Gβγ, Rictor and AKT as potential P-Rex1 interactors, and addressing the phosphorylation of mTORC1 and mTORC2 substrates. The integrity of these signaling complexes was investigated in P-Rex1 knockdown cells. Also, their regulation was studied by pharmacological inhibition of mTOR. In parallel, different P-Rex1 constructs were used to characterize the structural basis by which this multidomain RacGEF assembles signaling complexes including Gβγ, PI3K and components of mTORC2.
Our results indicate that proteins of the Gβγ-PI3Kγ-mTORC2-AKT pathway interact with P-Rex1 using this multidomain RacGEF as a plausible signaling platform and effector that regulates the spatiotemporal dynamics of the actin cytoskeleton, controlling cell migration.

Antiproliferative effect of white and brite adipocytes co-culture with lung cancer cells A549

Dulce María Caraveo Gutiérrez, Alicia Irasema Mendieta Trejo, Jesica Esther Escobar Cabrera, María Guadalupe García Alcocer, *Laura Cristina Berumen Segura. Universidad Autónoma de Querétaro, Cerro de las campanas S/N, Querétaro, Qro., México. Contact: (442)1921200 ext: 5530, lcbsq@yahoo.com

Cancer and obesity are considered major health problems in Mexico and worldwide. Cancer causes 8.2 million of deaths around the world; among cancer types, lung, breast, colon and liver have the greatest morbidity and mortality rate. In Mexico, the population that suffers lung cancer represents 8.4% of all deaths by cancer. On the other hand, Mexico has the second place with a major number of obese people. The relationship with these two diseases are known and the principal connection is the major component of adipose tissue, the adipocytes, that interact with cancer cells to promote a metabolic sustain within the microenvironment for the development of cancer. In the human body two types of adipose tissue exist, the white and brown, containing 3 types of adipocytes: white and brite in case of white adipose tissue and brown for a brown adipose tissue.

This study showed the proliferative effect of transwell co-cultures of lung cancer (A549 cell line) with human fibroblasts (HDFn cell line) and preadipocytes (3T3 cell line) after 48 hours and, contrary to what have been demonstrated previously we observed an increased rate of proliferation in the A549 cell line. This research also showed the effect of two adipocyte types (white and brite) derived of the previously mentioned cell lines.

The association of both diseases has been previously shown by other research groups, specifically at the interaction between cancer types that have a direct contact with adipose tissue, like ovarian, breast and prostate cancer, as well as, the promoting factors of this process. The aim of this study was to explore the relationship between lung cancer and adipose tissue, understanding the link between the cytokine secretion and growth factors like tumor necrosis factor α (TNFα), leptin and NFκB, to identify their participation in this process; and for the first time, to understand the role of the different types of adipocytes (white and brite) and their mechanisms for the increase of the proliferation of cancer cells (hormonal and metabolic effects).

Aquaporins in *Saccharomyces cerevisiae* potentially presents a differential role in the regulation of cell volume.

Chávez-Vega A+1*; Ledezma Guevara Daniela+1*; Uribe-López Jennifer A+1*; Morales-Tlalpan V2; Ferriz-Martínez RA2; Chávez-Servín J2; García-Gasca T2 & Saldaña C1,2
+These authors contributed equally. * Bachelor in Microbiology. 1Membrane Biophysics Lab. Basic and Applied Microbiology Unit. 2 Faculty of Natural Science. Autonomus University of Querétaro. C.P. 76140. carlos.saldana@uaq.mx

Aquaporines are tetrameric channels in which each of the monomers has a single pore domain through water molecules are transport. This displacement is bidirectional, determined by the electrochemmical gradient of the water. The size of these channels form 250 to 300 amino acids that are organized into six α-helix domains . One of the most interesting properties, which gives the name to these aquaporins, is the impediment of the to mobilize ions that, due to their size, could easily permeate througt their pores.
In *Saccharomyces cerevisiae*, two aquaporines have been found: AQY1 and AQY2, which differ from other species only by a transmembrane domain. The data basis of the genom of *S. cerevisiae* reveals that its aquaporines are homologous to the aquaporins of *Homo sapiens*. In adittion, a change in the volume of the yeast has been demonstrated when it is exposed to a osmotic shock; it is also return to its normal state once the original conditions return. In parallel studies, it has been shown that *S. cerevisiae* in the presence of hyperosmotic shocks is able to return to its original volume after 30 minutes. This is possible through the activation of the HOG signalling pathway, which culminate with glycerol biosynthesis. Previous studies in our laboratory show very fast and drastic changes in cell volume (up to 60%), in response to osmotic challenges.
In the present study, we show our preliminary results where we identify that the aquaporins of the yeast may have a differential role. That is, the aquaporin AQY1 works in shrinkage, while AQY2 does in swelling. Also, we show that both aquaporins can be partially inhibited wiht Hg (mercury) in micromolar concentrations. This was determined in studies made in a single mutans of each aquaporins.
In conclusion, our results open the possibility of understanding the molecular mechanisms of each yeast aquaporin, allowing the generation of a heterologous experimentation system, in which we can express aquaporine of other species and perform: 1) pharmacological studies with rapid results, 2) expressing aquaporins related to diseases of biomedical importance.
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PKCz involvement in the HIF protein levels regulation through GSK3b during experimental renal carcinogenesis

Patricia Curiel-Muñiz, José Solano, Francisco Aguilar-Alonso, Telma Pariente-Pérez, María Elena Ibarra-Rubio*.
Departamento de Biología, Edificio F, Laboratorio 225, Facultad de Química, UNAM, México, D.F. 04510. Tel. (5255)56223869, qfbpatriciacuriel@hotmail.com, meir@unam.mx*

Renal cell carcinoma (RCC) has a high mortality, its initial diagnosis frequently occurs at advanced or metastatic stages and the study of the first phases is virtually impossible in patients. Ferric nitrilotriacetate (FeNTA)-induced RCC model is a useful tool to analyze biological events at different points of the carcinogenesis process *in vivo*. We reported that experimental and human tumors are histologically identical, and identified that different early stages of carcinogenesis are generated after one and two months of FeNTA treatment.
We have determined that there are no changes in the renal amount of HIF-1a and HIF-2a at the first month of carcinogen treatment, whereas at the second one nuclear presence and total levels of both isoforms augment, as well as in neoplastic tissue. Also, p-GSK3b (Ser9) protein levels increase during experimental renal carcinogenesis and in tumors, which is consistent with the rise of PKCz renal levels. It is known that PKCz phosphorylates GSK3b on Ser9 and targets it for degradation via ubiquitination, leading, in turn, to HIF stabilization, since GSK3b phosphorylates HIF to be destroyed. On that basis, we analyzed the possible association of PKCz-GSK3b at different phases of the FeNTA model in the present work.
Our results demonstrated the co-immunoprecipation of PKCz and GSK3b, and it was observed at both early carcinogenesis stages studied as in tumors, implying that PKCz phosphorylates GSK3b preventing its function, which correlates with the increase of HIF protein levels.
In conclusion, these findings point to PKCz as responsible, at least in part, of the post-translational downregulation of GSK3b and as a probable cause of the HIF stabilization and enhancement from early stages of cancer development and until tumor maintenance in experimental renal carcinogenesis. This suggests the participation of the alterations observed in the cell malignization process, and they may be involved in the human disease as well, which would be important to investigate.
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Identification of the Guanine Nucleotide Exchange Factor VAV1 as a Novel Target of the Protein Tyrosine Phosphatase 1B.

Olga Villamar Cruz[1], Susana Monserrat Patiño Lémus[1], Marco Antonio Loza Mejía[2] and Luis Enrique Arias Romero[1]

[1] Unidad de Investigación en Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México. Av. de los Barrios #1, Los Reyes Iztacala. Tlalnepantla, Estado de México, Mexico. C. P. 54090. email: larias@unam.mx

[2] Facultad de Ciencias Químicas, Universidad La Salle Campus Ciudad de México, Benjamin Franklin 47, Cuauhtémoc, México 06140, México

ABSTRACT

PTP1B is a classical non-transmembrane protein tyrosine phosphatase that plays a key role in metabolic signaling and is a promising drug target for type 2 diabetes and obesity. Mounting evidence also indicates that PTP1B is overexpressed in colon, breast and ovarian cancers, and plays an unexpected positive role in HER2 signaling. In addition, experiments using cultured cells and mouse models of breast cancer have yielded conflicting results regarding the identity of the key substrates of PTP1B that mediate its positive role in HER2 driven transformation.

In order to identify possible molecular targets of PTP1B that mediate its positive role in HER2 positive breast cancer cells, we undertook a SILAC-based strategy, followed by phospho-peptide enrichment, and quantitative MS. This proteomic approach allowed us to identify VAV1, a tyrosine phosphorylated-regulated Rho guanosine nucleotide exchange factor (GEF) that participates in various cellular responses including actin cytoskeleton reorganization, gene transcription, and development and activation of immune cells, as a novel substrate of PTP1B.

Molecular docking studies revealed stable interactions between the PTP1B catalytic domain and VAV1. In addition, *in vitro* phosphatase assays confirmed that a phosphopetide corresponding to the residues 137-147 of VAV1 is dephosphorylated by PTP1B at Tyrosine residue 142. Finally, co-immunoprecipitation and co-localization experiments showed that PTP1B interacts with VAV1 in a cellular context, suggesting novel roles of this phosphatase in the regulation of several cellular processes including gene transcription, migration, survival and proliferation.

Activation of the MAP Kinase Pathway by Testosterone and DHT is Dependent on Src/EGF-R but Independent on the Intracellular Androgens Receptor in C2C12 Muscle Cells

Dennys Paola Ferreyra-Picazo, Fernanda Elizabeth Zúñiga-Aragon and Jesús Alberto Olivares-Reyes* CINVESTAV-IPN
*Corresponding Author: J. Alberto Olivares-Reyes Ph.D., Department of Biochemistry, CINVESTAV-IPN, Av. IPN 2508, Col. San Pedro Zacatenco, Mexico City, CP 07360 Mexico. E-mail: jolivare@cinvestav.mx, Phone 5255-5747-3951; Fax 5255-5747-3391

Recently, it has been found that the effects of androgens can be mediated by two different mechanisms of action, the classical pathway and the non-classical actions of androgens. In the "classical pathway", androgens bind to their cytosolic androgens receptor (ARs), member of the nuclear receptor superfamily that function as ligand-activated transcription factors. Once activated, these receptors bind to DNA and activate the expression of target genes. In the "non-classical" or non-transcriptional pathway, it is proposed that androgens bind to receptors on the plasma membrane and induce the activation of intracellular signaling cascades, such as activation of MAP kinases. Experimental evidence suggests that the membrane receptor could belong to the family of G-protein-coupled receptors (GPCRs); however, the precise nature of the membranal androgen receptor remains controversial, as well as the mechanism by which the ERK1/2 pathway is activated in muscle cells C2C12. Thus, this study aims to determine the non-classical signaling pathway involved in the activation of ERK1/2 by testosterone or dihydrotestosterone (DHT) in C2C12 cells and evaluate if those responses are dependent of the intracellular AR or through an AR located in the membrane. Our data suggest that testosterone and DHT activate the mitogen-activated protein kinase via Src kinase and the epidermal growth factor receptor by a mechanism that involves a receptor other than cytosolic AR. Interestingly, we also found the involvement of a G_i protein, since when using pertussis toxin we observed a decrease in ERK1/2 phosphorylation activated by testosterone and by DTH. However, we still have no evidence of how these responses are initiated. Our results and literature data suggest that the nature of the androgen-induced signal may depend on the target cell type, location, and type of receptor and ligand itself.

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Effect of Human Serum on IR/Akt/p70S6K Signaling in Breast Cancer Cells

Laura C. Flores García, Eric Alejandro García López, José Luis Ventura Gallegos y Alejandro Zentella Dehesa
Unidad Periférica del Departamento de Medicina Genómica y Toxicología Ambiental del Instituto de Investigaciones Biomédicas UNAM, Unidad de Bioquímica del Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán
Universidad Nacional Autónoma de México
Vasco de Quiroga No. 15 Col. Belisario Domínguez Sección XVI, Ciudad de México C.P. 14080, 54870900 ext. 2606
azentell@iibiomedicas.unam.mx

Breast cancer worldwide is considered a public health problem that represents the main cause of death from malignant tumors in women, representing 23 % of all neoplasms in the female population (Beltrán, 2014). One of the risk factors associated with the occurrence of breast cancer is obesity, which increases the incidence rate in 30 to 50 % of the development of the disease in this population (Aguilar et al, 2012). One of the points that allows to relate obesity to breast cancer is the alteration of the signaling pathway PI3K/Akt/p70S6K, which controls important aspects such as growth, proliferation, survival and motility; this signaling pathway is regulated by molecules such as insulin, IGF, estrogen, leptin, adiponectin, among other molecules that are deregulated in the obesogenic process. For this reason, the design of this study is based on the premise that differences in the serum composition of eutrophic women will have a differential profile in the activation of Akt signaling in breast cancer cells compared to the serum of obese women. To corroborate the above, the activation of the signaling pathway AMPK/Akt/p70S6K was evaluated in breast cancer cells that were exposed to the serum of eutrophic women and with some degree of obesity. The data obtained show a delay in the signaling of the IR/Akt/p70S6K pathway in cells treated with human serum will be compared to activation by insulin, starting at the Akt level which affects downstream molecules such as p70S6K; also observing the change in the molecular weight of molecules like AMPK, Akt and p70S6K in the extracts of cells exposed to human sera, which present differential patterns affected by the BMI and the menopausic state of the woman. In addition to the change in molecular weight of the molecules described above, different protein patterns were also observed at the isoelectric point and molecular weight, and specifically for the case of Akt was observed to decrease in the size and intensity of the dot obtained, which could be associated with the degradation of Akt. With the above we can conclude that the activation of the signaling pathway of IR/Akt/p70S6k is modified by the serum composition present in the serum of eutrophic women vs the serum of women with obesity, which could be associated with the increase in the incidence of breast cancer in the population of women with obesity.

Resistin induces an epithelial to mesenchymal transition-like process in mammary epithelial cells MCF10A

Octavio Galindo Hernández*[1], Raúl Díaz Molina[1], Mario Israel Oregel Cortez[1]
[1]Departamento de Bioquímica, Facultad de Medicina, Universidad Autónoma de Baja California, Mexicali, Apartado Postal 21100, Baja California, México. *Corresponding autor: Octavio Galindo Hernández, 1Departamento de Bioquímica, Facultad de Medicina, UABC, Dr. Humberto Torres Sanginés S/N, Centro Cívico, 21000 Mexicali, B.C.; phone: 686 557 16 22 ext: 122, E-mail: octavio.galindo@uabc.edu.mx.

Abstract

The epithelial-mesenchymal transition (EMT) is a biological process in which the epithelial cells progressively acquire mesenchymal features, including migration, invasion and a fibroblast-like phenotype. EMT is an key biological process involved in embryonic development, wound healing, fibrosis and cancer metastasis (1). In this process, epithelial cells progressively acquire a mesenchymal phenotype, and it is characterized by a decrease in E-cadherin, loss of apical-basal polarity, cytoskeleton reorganization, an increase in N-cadherin and vimentin levels, as well as matrix degradation and an enhanced ability for cell migration and invasion (1). In the modulation of the EMT process, the participation of non-receptor tyrosine kinases, including FAK and Src, in the regulation of migration and cellular invasion processes has been demonstrated (1). The World Health Organization (WHO) defines obesity as an excessive accumulation of fat that can be harmful to health, and several epidemiological studies support the relationship between obesity and the development of different types of cancer (2). Resistin also known as adipose tissue-specific secretory factor (ADSF) or C/EBP-epsilon-regulated myeloid-specific secreted cysteine-rich protein (XCP1) is a cysteine-rich adipose-derived peptide hormone and has been related to mammary tumor progression (3), however, the role of resistin as modulator of the EMT process has not been studied. In our present study, we show that resistin induce a downregulation of E-cadherin expression, which is accompanied with an increased expression of mesenchymal marker including N-cadherin, an increase of MMP-2 and MMP-9 secretion, the activation of FAK and Src, as well as cell migration and invasion in MCF10A cells. In summary, these findings demonstrate, for the first time, that resistin induces an EMT-like process in human mammary non-tumorigenic epithelial cells MCF10A.

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EXPRESSION OF TF ANTIGEN AND MOESIN IN MCF-7 CELLS STIMULATED WITH LPS

Luis Miguel García Cruz 1, Jesús Hernández2, Belem Gallegos2 y Pedro Antonio Hernández Cruz 2
1 Instituto Tecnológico de Oaxaca-Tecnológico Nacional de México, Av. Ing. Victor Bravo Ahuja #125 esq. Calz. Tecnológico. C.P.68030. Oaxaca de Juárez, Oaxaca, nutriologoluismiguel@gmail.com, 0449515706811, 0449513096312.
2 Laboratorio de genómica, proteínica y Glicobiología del cáncer, Facultad de Medicina y Cirugía, Universidad Autónoma Benito Juárez de Oaxaca, Ex hacienda de Aguilera s/n, San Felipe del Agua, C.P. 68020 Oaxaca de Juárez, Oaxaca, fuegoblanco136@yahoo.com.mx

INTRODUCTION: Infiltrating ductal carcinoma is the most common in women who have some alteration in the breast tissue. It has been found that in the majority of the cells of carcinomas there is an incomplete of the elongation oligosaccharide chains, forming structures less complex as the antigens TF (Galβ 1,3GalNAcα1-O-Ser/Thr) and Tn (GalNAcα1-O-Ser/Thr), which are prematurely linked with acid sialic, these antigens are associated with the uncontrolled growth of cells and the evasion of the immune system by the cells which express. These antigens are expressed in various, type mucin glycoproteins present in the membranes of MCF- 7 cells and may be responsible for the accession and cellular transformation. The TF antigen is recognized specifically by the lectin *Amaranthus leucocarpus* (ALL), which binds to a ~70 kDa glycoprotein on the surface of murine T cells with 41% identity with the moesin protein, which has a involvement in the epithelial-mesenchymal transition in different types of carcinomas, including mammary carcinoma. The exposure of tumor cells to LPS is associated with increased angiogenesis, vascular permeability and invasion of this type of cells, modifying their glycososylation profile, in addition, exposure with LPS in other cell lines has been linked to increased Moesin expression, however there are no studies that relate the increase of the expression of moesin and the modification of the glycosylation profile by stimulus with LPS in breast cancer cell lines. OBJECTIVE: Study the expression of TF antigen and moesin in cells MCF-7, stimulated with LPS. METHODOLOGY: MCF-7 cells are grown in RPMI-1640 supplemented with 10% inactivated fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 mm L-glutamine and incubated at 37 °C with 5% CO2. Cytochemistry 300.000 cells are deposited on a plate with six wells and grown during 24; plates were washed three times with PBS. The plates were incubated with Amaranthus leucocarpus lectin (ALL) labeled with FITC at concentration of 5 µg/ml and Anti-Moesin Ab during 2 and 4 hours, after; the plates were washed with PBS. Some tests were performed with LPS stimulation at a concentration of 20 ng/ml. Plates were observed with AXIOSCOP 40 microscope equipped with digital camera AXIOCAM MRC and micrographs analyzed with ZEN pro 2011 Software. Some experiments were carried out for flow cytometry; using a flow cytometer (Attune Nxt life technologies) and data were analyzed with the software of cytometry M Attune Nxt version 2015. RESULTS: There is an expression of TF antigen recognized by ALL in MCF-7 cells, as well as moesin, TF antigen expression remains unchanged when cells are stimulated with LPS, while expression of moesin increases. There is a colocalization of moesin and TF antigen in MCF-7 cells stimulated and not stimulated with LPS. CONCLUSIONS: The expression of moesin and TF antigen is altered in MCF-7 cells when stimulated with LPS, however there is a colocalization between these molecules, the moesin being able to be TF antigen binding protein.

Caracterization of LjROPs interactome change in *Lotus japonicus* roots upon inoculation by symbiotic bacteria *Mesorhizbium loti*

Autores: Ivette García Soto, Alexandre Tromas[1]
Centro de Ciencias Genómicas, UNAM
[1]Centro de Ciencias Genómicas, UNAM Campus Morelos Av Universidad S/N Col CHAMILPA. APDO. POSTAL 565-A 62210 Cuernavaca, MEXICO. Tel: (777) 3115164
alexandre.tromas@gmail.com

Legumes have developed the capacity to establish symbiotic interactions with soil bacteria. The endosymbionts, rhizobia, fix dinitrogen from the atmosphere and proportionate it in assimilable forms to the plant, in exchange for photosynthates. It has been established that accommodation of micro-endosymbionts inside the plant cell is an active process that requires proper cytoskeleton rearrangement. The Rho-GTPases (named ROP in Plants), considered as “molecular switches”, are known to activate the rearrangement of actin filaments during pollen tube growth and a role in symbiotic bacteria entry has been recently pointed out. ROP proteins interact with several partners, like activators, deactivators or effectors. Modification of ROP interactome in response to symbiotic bacteria still needs to be investigated to identify the signaling modules involved in this process. To do so, the legume model, *Lotus japonicus* will be transformed by a FLAG-tagged version of ROP to realize pulldown assays followed by liquid chromatography and double masse spectrophotometry (LC-MS-MS). The lists of proteins identified before and after inoculation with rhizobia will then be compared to determine which ones are recruited during the establishment of nitrogen fixing symbiosis.

Regulation of the Wnt/β-catenin pathway in hepatocellular carcinoma by an adenosine derivative

Nuria Guerrero-Celis, Jesús Rafael Rodríguez-Aguilera, Rebeca Pérez Cabeza-de-Vaca, Gabriela Velasco-Loyden and *Victoria Chagoya de Sánchez
Instituto de Fisiología Celular, Departamento de Biología celular y del desarrollo, Universidad Nacional Autónoma de México.
Cto. Exterior s/n, Ciudad Universitaria, 04510 Ciudad de México, CDMX
* vchagoya@ifc.unam.mx tel. 56225614

Introduction

Hepatocellular carcinoma (HCC) is the most common form of liver cancer of diverse etiology, usually associated with a state of cirrhosis. It occupies the first places in the incidence worldwide, showing a high level of mortality. It has been described that several molecular pathways are involved in the pathogenesis of HCC including Wnt/β-catenin. This pathway is very studied in cancer because it plays an important role in the development and hepatic regeneration. It has been described in 40% of the HCC this pathway is unregulated.

In the laboratory an adenosine derivative compound, called IFC-305, has been developed and it has shown an hepatoprotective effect inhibiting cell proliferation in an induction model of carcinogenesis with diethylnitrosamine (DEN).

Objective

To determine how IFC-305 regulates the Wnt/β-catenin signaling pathway in human hepatocellular carcinoma cell lines.

Method

Human liver cell lines (HepG2 and Huh7) were exposed to different concentrations of IFC-305 [0,10uM, 100uM, 500uM, 1 y 5 mM] during 48 hours to determine the cytotoxicity by sulforhodamine B staining. An immunofluoscence assay against β-catenin was performed in order to identify the subcellular localization of this protein in response to hepatoprotective compound.

Results

The cytotoxic effect was find with IFC-305 1mM and 5 mM in HepG2 and Huh7 cell lines, where there is a decrease in cell number compared to the control, cyclohexamide [50 ug/mL] was used as a positive control. Preliminary data suggest IFC-305 regulates the Wnt/β-catenin pathway by relocating β-catenin protein inside the cell, suggesting an inhibition of the “turn on” of the pathway. This behavior correlates with decrease in proliferation of cancer cells.

Conclusion

Exposure of liver tumoral cells to IFC-305 triggers a decrease in culture cells number which possibly is regulated by modulation of Wnt/β-catenin pathway. This founds contribute to the understanding of antitumoral effect of this hepatoprotective compound.

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IL-2 rescues peripheral blood mononuclear cells from apoptosis induced by cervical cancer cells

*Adriana Gutiérrez-Hoya**, Itzel Salazar-Valencia, Octavio Zerecero-Carreón, Arturo Valle-Mendiola, Benny Weiss-Steider and Isabel Soto-Cruz.*
Laboratorio de Oncología Molecular, FES Zaragoza, Universidad Nacional Autónoma de México, Batalla 5 de mayo s/n Col. Ejército de Oriente, CP 09230, Ciudad de México, México.
**Investigador CONACYT. Tel: 56230796, adrianagh85@hotmail.com

Cervical cancer is one of the world's deadliest, is the fourth most common cancer in women. There were an estimated 530,000 new cases of cervical cancer in 2012, this represent 7.5% of female cancer mortality. Almost nine out of ten (87%) cervical cancer death occur in the developed countries, such as México. In the developed countries, the limited access to effective cervical cancer screening and HPV testing increases the mortality rate since the cancer is not detected until the symptoms appear in the more advanced phases. Additionally, the expectation of treatment is not good in advanced stages of the disease, for this reason the mortality rate for cervical cancer is much higher than originally estimated (52%). Therefore it is necessary to study new molecules than can eliminate the tumour cells and that can activate the immune system. In this context our working group has found that high concentrations of IL-2 (Lymphocyte grown factor), promote the immune system activation and inhibited the growth of cervical cancer cells. Also, that the JAK/STAT pathway is active in cervical cancer cells. To understand the signaling pathways that are activated by IL-2 in cervical cancer cells that induce apoptosis and its relationship with normal immune cells we analyzed the effect of high concentrations of IL-2 in co-cultures of cervical cancer cells and normal heterologous peripheral blood lymphocytes.
Methodology: Normal peripheral blood mononuclear cells (PBMC) were obtained by density gradient centrifugation using ficoll-paqueTM. The cervical cancer cell lines HeLa, INBL, CaSki and C33A were co-cultured with the PBMC in a 1:10 ratio for 48 hours in the presence of different concentrations of IL-2. After this time the cells were stained with annexin V-PE and propidium iodide (PI) or 7AAD and analyzed by flow cytometry.
Results: We observed that co-culturing cervical cancer cells with the PBMCs in the presence of low doses of IL-2 induced apoptosis in PBMCs (42%) and in cervical cancer cells (35.5%).
The opposite, the co-culture of cervical cancer cells with the PBMCs in the presence of high doses of IL-2, rescued the PBMCs from apoptosis (35%) this percentage of apoptosis is similar to basal values (32%).
Conclusion: The cervical cancer cells are able to induce apoptosis in PBMCs. Nevertheless high doses of IL-2 can rescue the PBMCs from undergoing apoptosis. It is possible that IL-2 is activating the JAK/STAT pathway to induce cell survival.

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Capsaicin Inhibit both force production of isolated skeletal muscle and physical performance of intact mice in an administration route dependent manner.

Ana Maria Guzman Ambriz (graduate student), Miguel Huerta Viera[1], Mónica Ríos Silva[2], Xóchitl Trujillo Trujillo[1], Adán Dagnino Acosta [1],*.
[1]. Universidad de Colima, Centro Universitario de Investigaciones Biomédicas, col. las víboras, calle; 25 de julio #965. CP. 70228.
[2]. CONACYT-Universidad de Colima. Centro Universitario de Investigaciones Biomédicas, col. las víboras, calle; 25 de julio #965. CP. 70228. *dagninoa@ucol.mx, anita27n@hotmail.com

Capsaicin is the active element of chili peppers broadly recognized as an agonist of TRPV1 receptors. Additionally, this drug has been proposed as an agonist for cannabinoid receptors (CB1). Capsaicin administration in intact animals and humans shows metabolic and functional effects in different tissues of the organism, at the level of skeletal muscle, the effect of capsaicin is still not clear. In fact, intraperitoneal administration of capsaicin (acute or chronic) improves the physical performance of rodents. In contrast, acute exposition of isolated skeletal muscle to capsaicin produces a decrease in muscle force generation.
The goal of this study was to determine the source of these apparently opposite results and additionally identify the target of capsaicin that accounts for the reduction in the produced force of the isolated muscle.
The effect of capsaicin was studied in C57BL/6J mice of 6-8 weeks old. The force generated in intact mice was evaluated using the grip strength meter with a four paws grid. For direct studies in muscle, the EDL (fast) and soleus (slow) muscles were isolated and maintained immersed in an organ chamber filled with solution at 37°C and CO_2/O_2 bubbling. The muscle was electrically stimulated with square pulses applied at different frequencies. The studies in individual fibers were performed with isolated FDB (fast) muscles enzymatically digested. The calcium kinetic determination was performed using Mag-Fluo4 loaded in isolated FDB fibers. Statistical differences were evaluated using a student's *t*-test with a p value <0.05.
According to published results, our data show that mice acutely administered with intraperitoneal capsaicin developed a 15% increase of force after 3 hours of the administration. Conversely, the subcutaneous administration of capsaicin above the gastrocnemius region generated a significant decrease of force 30 minutes after the injection, in line with this finding, the isolated EDL muscle exposed for 20 minutes to capsaicin showed a decreased sensibility to voltage stimulation. The isolated fibers loaded with a Mag-Fluo4, a low affinity calcium sensor showed a significant reduced response specially at high frequencies of stimulation. Finally, the recovery constant of the calcium transients showed no significant difference in fibers incubated with capsaicin.
In conclusion, our results reproduce the inhibition of muscle force obtained in isolated muscle by modifying the administration route of capsaicin. This drug inhibits the force production in isolated EDL muscle and reduces the calcium released during a high frequency of electrical stimulation. Support: CONACYT PN-2016/2120 to ADA.

Structural and functional analyses of mammalian sperm-specific Na+/H+ Exchanger (sNHE) imply that the mammalian sNHEs are differently regulated according to species.

SANDRA HERNÁNDEZ-GARDUÑO[1], JULIO CESAR CHÁVEZ ZAMORA[1], AND TAKUYA NISHIGAKI[1].
[1]*Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México*
Cuernavaca, Morelos 62210, México. +52-7773-291709. takuya@ibt.unam.mx

Intracellular pH (pHi) plays an important role in sperm motility. In 1980s, Lee reported in sea urchin spermatozoa the existence of an atypical Na^+/H^+ Exchanger **(**NHE), which is activated by hyperpolarization of the membrane potential (Em) (Lee. 1983. Develop Biol. 95:31-45). In 2003, sperm-specific NHE (sNHE) was identified as an essential protein for male fertility in mice (Wang et al., 2003. Nat Cell Biol. 5, 12:1117-1122). In accordance with the report of sea urchin, the sNHE contains a consensus sequence for a voltage-sensing domain (VSD). Actually, it was recently demonstrated that Em hyperpolarization can increase the pHi in the mouse spermatozoa probably through sNHE. In contrast, it has been unclear if sNHE of human spermatozoa responds in a similar way as those of mouse. The presence of voltage-gated H^+ channel in human spermatozoa, but not in mouse spermatozoa, also implies a possible difference of sNHE activity among the species in mammals. Therefore, we first compared the amino acid sequence of the putative VSD of sNHE of different mammalian species in order to obtain structural properties of sNHEs. The analysis of 73 species of mammalian reveals that most of species have a typical segment 4 (S4) of known VSDs, namely 4 positively charged residues every three amino acid (RRRK). However, the S4 of most of primates and few non-primate mammals contains only three positively charged residues (mainly QRRK or RRPK). Taking this information into account, we are currently trying to determine the regulation of the activity of sNHE using several mammals including mouse, human and pig. The sNHE activity can be determined by extracellular Na^+ dependent intracellular pH increase (pHi measurement with fluorescent indicator such as SNARF-5F). Also, a recover of sperm motility in a medium containing sodium acetate that acidifies pHi can be an alternative technique to estimate an increase in pHi. Our preliminary results support the functional differences of sNHE between mouse and human.
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Evaluation of the protein expression and localization of Retinoblastoma mutants.

Jesús Hernández-Monge, Adriana B. Rousset R., Vanesa Olivares I.
Instituto de Física, UASLP. jesus_hernandez_m@hotmail.com

The study of the tumor suppressor Retinoblastoma (Rb) protein, since its discovery 29 years ago, has been focused in the regulation of the cell cycle progression. Through all these years the Rb pathway have been found altered, somehow, in virtually every cancer type. The Rb protein and its family members are components of a canonical pathway that mediates cellular responses of a variety of stress signals. Rb controls the transcriptional activity of E2F and their target genes, needed for the cell cycle progression. When Rb protein is hyper-phosphorylated by the cyclin-dependent kinase complexes (CDKs) become inactive and dissociates from the E2F transcription factor. Despite the abundance of reports that associate RB gene mutations with Retinoblastoma, it is not known the underlying mechanism loosen by these point mutations. In an attempt to board this matter, we aimed to analyze the expression and localization of some RB mutants by transient transfection of constructed mutants of Rb in the H1299 cells, where the RB gene was knocked down using the CRISPR/CAS9 method.

Impact of *O*-GlcNAcylation over the PI3-kinase/Akt pathway in Oral Squamous Cell Cancer

Daniela Illescas Barbosa[1], Victoria Jiménez Castillo[2],[3], Hugo Sánchez Martínez[2], Risk Diaz Castillejos[2], Cynthia Diaz Hernández[1], Anayetzin Torres Rivera[1], Beatriz Xóchitl Ávila Curiel[2], Fabián Tafoya Ramírez[3], Rafael Torres Rosas[2], Tony Lefebvre[5], Marlène Mortuaire[5], Carlos Josué Solórzano Mata[1,2]*. [1]Faculty of Medicine and [2]Faculty of Dentistry, Autonomous University Benito Juárez of Oaxaca, [3]Technological Institute of Oaxaca, [4]HRAEO Departament of Pathology, Mexico; [5]Université de Lille 1 CNRS/UMR-8576-UGSF Lille, France.
Email: universidad99@hotmail.com

Oral squamous cell carcinoma (OSCC) is a public health problem with an increased incidence in young people. In the OSCC is expected that alterations in glycosylation could modify signaling pathways that promote tumor progression. *O*-GlcNAcylation is a dynamic, reversible posttranslational modification that involves the addition of GlcNAc to Ser/Thr residues of cytosolic, nuclear and mitochondrial proteins, catalyzed by the enzymes OGT and OGA. In several types of malignant tumors, enhanced *O*-GlcNAcylation triggers the growth, invasion and cell resistance to death. These consequences are currently unknown in OSCC and how this modification affects the regulation of the PI3-kinase/ Akt pathway remains undeciphered. The purpose of this work was to determine the level of the *O*-GlcNAcylation in OSCC tumors and healthy tissues, as well as the impact of this modification in the activation of the PI3-kinase/Akt cascade in the SCC-152 cell line.

Our results showed that OSCC tumors overexpressed the *O*-GlcNAc modification both in the cytoplasm and the nucleus, such as the SCC-152 cells. On the contrary, healthy tissues exhibited lower expression of the *O*-GlcNAcylation that is localized mainly in the cytoplasm. In SCC-152 cells, treatments that stimulated the *O*-GlcNAcylation, activated the PI3-Kinase/Akt pathway with an increase in Akt phosphorylation, however, significant changes were not observed in proliferation, although the modification promoted the morphological and structural integrity of the colonies. In conclusion, our results indicate that *O*-GlcNAcylation stimulates the PI3K/Akt pathway by Akt phosphorylation, and impacts the integrity of the colonies which could be related to the tumor growth and survival.

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Key words: Oral squamous cell carcinoma, *O*-GlcNAcylation, PI3-K/Akt pathway.
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The G-protein β subunit, Gpb1, regulates virulence in *Mucor circinelloides*

Marco Iván Valle-Maldonado, Nancy Yadira Reyes-Mares, José Alberto Patiño-Medina, Irvin Eduardo Jácome-Galarza, Rafael Ortíz-Alvarado, Martha Isela Ramírez-Díaz, Carlos Pérez-Arqués, Victoriano Garre, Víctor Meza-Carmen*
*Laboratorio de Diferenciación Celular, Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Ciudad Universitaria, 58030, Morelia, Michoacan, Mexico. victor_meza2004@yahoo.com.mx.

Heterotrimeric G proteins (HGP) are composed by Gα, Gβ and Gγ subunits, and they regulate signal transduction pathways in eukaryotic organisms. Thier participation in morphogenesi and virulence has already been described in fungi previously[1,2]. *Mucor circinelloides* is a dimorphic mucoral and human opportunistic pathogen[3]. Understanding of molecular regulators of virulence in this fungus remains scarce. A previous manuscript in our work group, reported the presence of 12 Gα, 3 Gβ and 3 Gγ subunits encoding-genes in *M. circinelloides* which is the largest repertoire in fungi[4].
Interestingly, a transcript evaluation was carried out for HGP encoding genes during dimorphism; and it revealed that *gpb1* had the highest transcript levels during mycelial growth. In silico evaluation of gpb1 promoter region revealed putative recognition motifs for transcription factors that regulate polarized growth in fungi. This might be relevant as filamentous growth is the virulent form in *M. circinelloides* as a result of its invasibility potential.

RESULTS. Firstly, *gpb1* gene interruption was carried out in the MU402 strain (leu^-, $pyrG^-$) by homologous recombination. A fusion PCR-obtained recombinant fragment was employed. The mutant *gpb1Δ* (leu^-, $pyrG^+$) was assesed by PCR and RT-qPCR. Spore size and germination rates were not different when compared to wild type strain. However, the mutation led to a disminished spore production (40%). Interestingly, the *gpb1* gene deletion reduced mycelial growth in comparison to wild type: radial growth (60%), primary hyphae (70%), and secondary hyphae (20%), and biomass production (10%). These results suggest a posible role in filamentous growth regulation by *gpb1* in *M. circinelloides.* The *gpb1* gene disruption led to the loss of virulence against murine model. Diabetic mice inoculated by *gpb1Δ* spores survived a seven-day survival assay. Half of mice infected by wild type sporres died in the same experiment. Invasibility of *gpb1Δ* and MU402 strains in murine liver was determined by qPCR, and it revealed that a significant decrease (80%) of fungal burden for the mutant *gpb1Δ* in comparison to wild type. This reduced invasion could be explained by the reduced filamentous growth deployed in the mutant. We are currently determining the relation of *gpb1* with other already characterised molecular regulators of filamentous growth in this fungus (PKA pathway).

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IL-2 induces an increase in the expression of autophagy-related markers in cervical cancer cells

María del Carmen Lagunas Cruz, Alejandra Zendejas García, Arturo Valle Mendiola, Benny Weiss Steider, Isabel Soto Cruz.
Laboratorio de Oncología Molecular L9PB, Unidad de Investigación en Diferenciación Celular y Cáncer (UMIEZ), Facultad de Estudios Superiores Zaragoza, UNAM. Avenida Batalla 5 de mayo esquina fuerte de Loreto S/N, colonia Ejército de Oriente, Iztapalapa, DF. Teléfono: 56230796.
lagunascruzmaryc@comunidad.unam.mx; sotocruz@unam.mx

Cervical Cancer is the second cause of death in women worldwide according to WHO. In Mexico the highest incidence in gynecological tumors is breast cancer and secondly cervical cancer. Several therapies have been used for the treatment of cervical cancer. For example, it has been demonstrated that the effect of IL-2 on non-hematopoietic cells appears to be differential since higher concentrations inhibit proliferation of squamous carcinoma head and neck cell lines, either *in vitro* or *in vivo.* We have found that low concentrations of IL-2 induce proliferation on cervical cancer cells; on the contrary, high concentrations induce inhibition of proliferation. The treatment of cervical cancer cells with 100 IU/IL-2 for 48 and 96 hours, diminishes cell proliferation, induces a low percentage of apoptosis and cells are arrested in G1-phase of cell cycle. This treatment does not induce senescence, thus it is possible that arrested G1 phase cells do not proliferate but instead promote autophagy for survival. However, there remain many important, unanswered questions about the exact mechanisms of autophagy mediated cancer suppression and promotion. To evaluate whether IL-2 induces an increase in the expression of autophagy-related markers in arrested cells to promote cell survival, HeLa and INBL cervical cancer cells were treated with 100 IU/ml of IL-2 for 48 and 96 hours. We analyzed the expression of autophagy-markers beclin-1, Lc3b and Atg5 by standard PCR. Lc3b was also analyzed by flow cytometry. The results showed an increase in the expression of beclin-1, Lc3b and Atg5 in cervical cancer cells after treatment with IL-2. The increase of autophagy-related markers suggests that this process is activated in response to IL-2. Some cancers induce autophagy and are dependent on autophagy for survival. Therefore, it is possible that IL-2 induces autophagy-mediated recycling to meet the elevated metabolic demand of growth and proliferation for survival of cervical cancer cells.

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Maize CycD2;2 and KPR4;2 distribute differentially along maize embryo axe during germination and its location is dependent on sugar and auxins.

Aurora Lara-Núñez, Diana Ixchel Romero Sánchez, Karina Durán Jiménez, Sonia Vázquez-Santana and Jorge M. Vázquez-Ramos.
Facultad de Química, UNAM. Laboratorio 114. Edificio E, Departamento de Bioquímica.
Facultad de Química, Ciudad Universitaria. Tel.55 56225284
Mail: auroraln@unam.mx

Germination is a determinant process in plant life cycle. A seed must imbibe water to activate metabolic processes and trigger the morphogenetic program that gives rise to a plantlet. Among the processes that take place, the cell cycle is critical for cell growth and further differentiation.

The cell cycle is a series of events that take place inside each cell that will allow proliferation in response to intrinsic and extrinsic signals to give rise to two daughter cells with equal DNA content. Among the extrinsic signals that a seed could be exposed to are light quantity, temperature and humidity; intrinsic signals could be the nutritional state or the presences of phytohormones.

Cell cycle progression is mainly regulated by the kinase activity of heterodimers formed by

cyclins (Cycs) and Cyc-dependent kinases (CDKs). In plants KIP-RELATED (KRP) proteins inhibit the CDK-Cyc complexes and control cell cycle progression. Cycs D and KRPs play a central role in balancing cell proliferation and response to mitogenic signals.

Maize CycD2;2 protein accumulates during progression of the cell cycle at early germination times, it is detected on nuclear and cytoplasm fractions, and responds positively to glucose. In contrast, the protein abundance of the inhibitor KRP4;2 is not affected by sucrose along germination.

We present here a closer analysis of the presence of both proteins, via immunolocalization, longitudinally along embryo axis during germination. A differential tissue and cellular pattern localization of CycD2;2 and KRP4;2 proteins were observed when axes were exposed to glucose, sucrose, auxins (IAA) or no sugar was added to the imbibition medium. Two imbibeition times, beside dry seed, were monitored: 18 and 36h.

Both proteins were present importantly along dry embryo axes. KRP4;2, without sugar, declined slightly at 18h to increase again at 36 h. By contrast, on presence of both sugars it declined gradually, more evident on Suc. On Glu, KRP4;2 accumulated in quiescent cells and around them (proliferating zone) at 18h; at 36h the accumulation took place mainly at primordium seminal root cells. On IAA there was a dramatic decline at 18h and a protein buildup in quiescent cells and around them, and in primordium seminal root cells at 36 h.

CycD2;2 was present along embryo axes without sugar, with a slight decline at 18h and then accumulation at 36h, like KRP4;2. On Suc, it disappeared on both times analyzed, while on Glu there was also a declined at 18 h. At 36 h a high protein buildup was observed in proliferating cell zone, above quiescent center and, at lower level, in leaf primordium and apical proliferating zone. On IAA a decline at 18 h was also observed along embryo axe, but at 36h the increase was evident in primordium seminal root cells.

Evidently, the presence of Suc, Glu or IAA triggers distinctively the morphogenetic program of embryo axes along germination which is reflected on differential protein patterns of cell cycle markers, mainly on proliferating zones.

Regulation of the expression of SnoN, a Negative Modulator of the TGF-beta pathway, in Hepatocytes.

David Martínez Pastor, Dra. Marina Macías Silva.
Instituto de Fisiología Celular, Lab. 225 Norte, UNAM.
Circuito Exterior s/n, Ciudad Universitaria, Coyoacán, 04510 México D. F.
Tel. +52 55 56-22-5729; e-mail: mmacias@ifc.unam.mx

SnoN is an inhibitor of the transforming growth factor-beta (TGF-β) signaling pathway that functions as cofactor for the transcriptional factors known as Smad. SnoN plays major roles in different physiological processes, and alterations in its expression are related with some pathologies. TGF-β regulates SnoN at both levels: gene transcription and protein stability. Regarding to protein stability, TGF-β promotes the degradation of SnoN through proteasome: active Smad2/3 complex recruit an E3 ubiquitin ligase such as Smurf2, APC or Arkadia, which ubiquitinate SnoN, leading to its degradation via ubiquitin-proteosome system (UPS).

Additionally, SnoN is able to undergo sumoylation, a process conducted by the E3 SUMO ligases PIAS1 and TIF1γ, modificating its function as transcriptional coregulator. However, SnoN stability is also regulated by changes on actin cytoskeleton dynamics by a mechanism independent of TGF-β. Thus, the polymerization of actin (F-actin) increases the stability of SnoN, although the molecular mechanism that controls this process remains unclear; in this respect, one hypothesis suggests that it could be through post-translational modifications like sumoylation or through activation of deubiquitinases (DUBs). The results show that modifications in the dynamics of the actin cytoskeleton (favouring the formation of F-actin) increase the levels of SnoN protein in C9 cells; furthermore, this increase in SnoN levels is TGF-β/Smad pathway activation-independent. Also, the modification of the polymerization and depolymerization patterns in the actin cytoskeleton has an effect on the ubiquitination state of the total proteins and specifically on SnoN. In addition, we demonstrate that there is an interaction between SnoN protein and actin, while the subcellular localization of SnoN changes after modifying the dynamics of the cytoskeleton, suggesting that this affects its function as a regulator of the TGF-β pathway.

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Effect of IL-2 on the activation and nuclear translocation of STAT5 in cervical cancer cell line SiHa

Diego Francisco Morelos Laguna, Arturo Valle Mendiola, Benny Weiss Steider, Octavio Zerecero Carreón, Itzel Salazar Valencia and Isabel Soto Cruz.

Laboratorio de Oncología Molecular L9PB, Unidad de Investigación en Diferenciación Celular y Cáncer, Facultad de Estudios Superiores Zaragoza, UNAM. Avenida de 5 de mayo, esquina fuerte de Loreto S/N, colonia Ejército de Oriente, Iztapalapa, DF. Teléfono: 56230796.
issocruz@yahoo.com; diego5morelos@gmail.com.

IL-2 is a crucial cytokine for the well-functioning of lymphocytes. The IL-2 receptor (IL-2R) is mainly found in lymphocytes. In normal conditions, the IL-2 triggers the activation of STAT5 to be able to migrate to the nuclei and promote the transcription of genes linked with cell proliferation and survival. However, some types of tumours express IL-2R, such as breast cancer, prostate cancer and cervical cancer. Our workgroup has demonstrated that HPV18 positive cell lines CALO and INBL express IL-2R and they proliferate in response to low concentrations but high concentrations inhibit their proliferation. At this point it is not clear whether tumour cells derived from a cervical cancer HPV 16+ have the same response. For this purpose, we used SiHa cell line derived from a cervical cancer HPV 16 positive. Approximately $1x10^6$ cells per condition were stimulated with 10UI/mL and 100UI/mL of IL-2 for 5, 15 and 35 minutes. We determined the phosphorylation of cytoplasmic and nuclear STAT5 by flow citometry. To evaluate cell proliferation, 1000 cells per well were seeded in a 96 well-plate. They were stimulated with 10UI/mL and 100UI/mL IL-2 for 24, 48, 72 and 96 hours. The proliferation rate was determined by crystal violet assay. The cells treated with 10UI/mL of IL-2 increased their proliferation rate, and a proliferation-decreased in the cells treated with 100UI/mL of IL-2. We observed a significant increase in the activation of STAT5 and its migration to the nucleus when the cells were stimulated with 10UI/mL of IL-2 for 35 minutes. When cells were treated with 100UI/mL we observed the opposite effect. These results are consistent with the effect of IL-2 on cells derived from HPV18 positive cervical tumours that enhance their proliferation rate and survive with low doses of IL-2, while opposite effects are observed with high doses. Our results suggest that these two types of papilloma virus, considered as high risk and found in almost all cervical tumours around the world, share the same mechanism to induce proliferation. IL-2 has been used as a treatment against cancer, but this treatment might be risky since low doses of IL-2 enhance proliferation of tumour cells, and the required doses of IL-2 to inhibit the proliferation rate of malignant cells are very toxic to the patients.

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Peroxisome and mitochondrial dynamics regulated by Dnm1 are necessary for sexual development in the fungus *Podospora anserina*

Raful Navarro Espíndola, Fernando Suaste Olmos, Leonardo Peraza Reyes

Instituto de Fisiología Celular, Universidad Nacional Autónoma de México. Circuito Exterior s/n. Ciudad Universitaria, Coyoacán, 04510 Mexico City, Mexico.
Tel. (55) 5622-5628. rasane18@gmail.com, lperaza@ifc.unam.mx

Fission is a mechanism of utmost importance for cell dynamics in most organisms. From great mammals to bacteria, fission accomplishes the division of two cell compartments by making use of molecular motors and adaptor proteins. One of the best known proteins involved in fission is the molecular motor encoded by the *DNM1* gene, Drp1 in humans, and its dynamin-like orthologs in other organisms. Deletion of these proteins often causes a phenotype where mitochondria and peroxisomes are completely fused and make networks or big masses along the cell that interfere with cell dynamics. In fungi, organelle dynamics are relevant for sexual development. Our lab has demonstrated that peroxisome dynamics, size and shape progressively change during sexual development in *Podospora anserina.* Furthermore, other studies have shown that depletion of peroxisome import proteins have effects, both in sexual development and in the form and dynamics of mitochondria. This evidence suggests that peroxisome and mitochondrial dynamics are tightly related and necessary for the correct progression of sexual development in *P. anserina*.

In this work we investigated whether the dynamics of mitochondria and peroxisomes are required for *P. anserina* sexual development by deleting the gene encoding the fission molecular motor PaDnm1. We first confirmed that elimination of PaDnm1 produced a phenotype of fused mitochondria and peroxisomes, consistent with participation of this protein in the fission of both organelles. In addition, we observed a delay in the growth and development of *Δdnm1* strains. Moreover, we found that these strains were able to reproduce sexually and develop ascospores. However, a large number of ascospores have abnormal morphology or contained irregular numbers of nuclei.

We conclude that the dynamics of mitochondria and peroxisomes are of great importance for sexual development, where they play important roles both in the segregation of the nuclear products of meiosis and in the formation of the meiotic-derived spores.

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Steroidogenic activity of fetal Leydig cells in rabbit

Alexis Paulina Ortega García1, Pedro Cuauhtémoc Tonatiuh Collazo Saldaña1, Adriana Castro Domínguez1, José Alejandro Marmolejo Valencia1, Verónica Díaz Hernández2, Horacio Merchant Larios1.
1Universidad Nacional Autónoma de México, Instituto de Investigaciones Biomédicas, Biología Celular y Fisiología, 04510, Delegación Coyoacán, D.F. Phone: 01555622-3833, Email: merchant@unam.mx
2Universidad Nacional Autónoma de México, Facultad de Medicina, 04510, Delegación Coyoacán, D.F.

Summary

In the human fetal testicle, the differentiation of the germinal lineage is an asynchronous process, dependent on the somatic context. It is carried out in close association with the Sertoli cells in the seminiferous cords separated from the stroma by a basal membrane that surrounds them. In the stroma, the irregularly distributed Leydig cells initiate their steroidogenic activity early and influence the gonocyte-Sertoli interaction processes. Given the complexity of the endocrine, paracrine and autocrine interactions that occur during fetal life in larger and prolonged gestation species, the rabbit represents a better experimental model than the mouse to know the role of Leydig cells during the early stages of the disease in testicular development of mammals.

Results

The highest expression level of the *SF-1* gene was at 17 dpc (post-coitus days), then decreased. Regarding the genes of *CYP11A1* and *3B-HSD*, the highest levels were at 21 dpc and one dpp (postpartum day) and the expression profile of AR (receptor to androgens) was low about the other genes with an increase in expression at 17 dpc, 27 dpc, and one dpp.

The confocal immunofluorescence technique revealed the presence of CYP11a1 in fetal and postnatal Leydig cells. We found that Leydig cells decrease their proliferative index as their ultrastructural differentiation progresses, which correlates with the ability to secrete steroid hormones.

Their larger size distinguishes the Leydig precursor cells compared to the other stromal cells. At the beginning of the ultrastructural differentiation, they accumulate lipid drops and the mitochondria increase in size while maintaining their laminar cristae. Afterwards, the Leydig cells continue abundant cisterns of the Golgi apparatus occupy the cytoplasm, and the smooth endoplasmic reticulum and the mitochondria acquire tubular crest, typical of the steroid hormone producing cells.

Conclusions

The expression profiles of the following factors were analyzed: The *SF-1*, *CYP11A1*, *3B-HSD* and the androgen receptor (AR). Leydig cells decrease their proliferative index as their structural differentiation progresses, which correlates with the ability to produce and secrete steroid hormones.

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In vitro studies of Nobiletin on FaDu cell line from hypopharyngeal cancer.

Gutiérrez-Venegas, Gloria; Contreras-Astudillo, Roberto; Ortiz-Miranda, Laura.

Laboratorio de Bioquímica, Facultad de Odontología, División de Estudios de Posgrado e Investigación, UNAM. * Corresponding author: Gutiérrez-Venegas, Gloria. Laboratorio de bioquímica, División de Estudios de Posgrado e Investigación de la Facultad de Odontología, Universidad Nacional Autónoma de México, Ciudad Universitaria, 04510, Ciudad de México, México. Phone (+52) (55) 5622-5554. Fax (+52) 5622-5554, email: gloria@fo.odonto.unam.mx

Abstract.

Cancer is one of the common diseases worldwide, in fact, each year, tens of millions of people are diagnosed with cancer around the world, and more than half of the patients eventually die from it. There are some risks factors, such as tobacco, alcohol, unhealthy diet and exercise, which can increase the probability to developing cancer.

Oral cancer is a highly relevant problem of global public health. According to Rivera, C. (2015), it is located within the top 10 ranking incidence of cancers and despite the progress in research and therapy.

On the other hand, flavonoids, a class of phenolic compounds that are found in fruits, vegetables and tea, among others, are now considered as an indispensable component in a variety of pharmaceutical, medicinal and cosmetic applications, due to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties. Nobiletin is a natural polymethoxylated flavonoid that can be isolated from citrus fruit peels and that has shown anti-carcinogenic properties in some cell lines, beside its anti-dementia activity.

This property has drawn our interest to research the effect of nobiletin on cancer cells obtained from hypopharyngeal cancer (FaDu cell line), in which we are focused on evaluate cellular viability, migration, cell signaling and Vascular Endothelial Growth Factor (VEGF) production.

E5 from HPV16 impairs the increased levels of cell cycle regulators and reverts the transforming state of Ha-Ras expressing cells.

Adolfo Pedroza-Saavedra, Vanessa Gomez-Gomez, Lilia Chihu-Amparan, Minerva Maldonado-Gama, Fernando Esquivel-Guadarrama, Lourdes Gutiérrez-Xicotencatl. Instituto Nacional de Salud Pública. Av. Universidad 655 Col. Santa María Ahuacatitlán, C.P. 62100, Cuernavaca, Mor., México. Tel. 777-3293000 ext. 2261, mlxico@insp.mx

The E5 protein from human papillomavirus (HPV) type 16 (16E5) is a low molecular weight hydrophobic protein proposed to be potentially oncogenic. It is not very well understood its function in cell environment and in transformation. Cell transformation mediated by 16E5 depends partially on the mitogenic stimulus of epidermal growth factor receptor (EGFR), through increasing the tyrosine kinase activity of it. Expression of 16E5 correlates with activation of components of the EGFR signaling pathway, such as MAPKs that regulates genes involved in cell growth. We demonstrated that 16E5 synergizes with EGFR to down-regulate $p27^{Kip1}$ (CKI) allowing cells to stay for a longer time into S phase of cell cycle and favoring initiation of transformation. 16E5 uses the EGFR pathway to transduce signals to the nucleus and Ras protein is important in this pathway. We have observed previously that 16E5 maintains the equilibrium between the Ras-controlled MAPKs and PI3K pathways, allowing the regulation of cell cycle and thus modulating cell proliferation and survival signals. In this study, we analyzed the effect of 16E5 on MAPKs and pI3K/AKT signaling pathways, and also cell cycle regulators.

For this, a fibroblastic cell line derived from an FVB mouse was transfected either with 16E5 and/or HrasG12V (activated Ras). Expression of 16E5 (RT-PCR) and Ras (Western blot, WB) were determined. Transforming ability of Ras, 16E5 or in combination was determined by cell growth curves and anchorage-independent assays; also, components of MAPKs and PI3K signaling pathways, and cell cycle regulators (cyclin A, cyclin D1, $p27^{Kip1}$, $p21^{Waf1}$, p16, p53, pRb) were determined both in the absence or presence of EGF and analyzed by WB.

Surprisingly, the results showed that expression of 16E5 reduced the ability of Ras to form transforming foci in agar up to 6.5 times (-EGF) and 2.2 times (+EGF). Activation of the EGFR pathway in the presence of 16E5 increases the SOS1 levels and promotes an apparent degradation of Ras. When co-expressed 16E5 reverts Ras tendency to decrease Raf levels as well as its activity and a similar effect is observed for MEK2. Ectopic expression of Ras results in augmented levels of cyclin A, cyclin D1, p53, and $p21^{Waf1}$, while those of FOXO3a, $p27^{Kip1}$, and p16 were decreased. However, when 16E5 and Ras were co-expressed the opposite was observed.

Cyclin D1 is necessary for progression at the G1 phase, but it has been reported that high levels of this protein are associated with transformation due to genomic instability. Possibly the higher levels of p53 and $p21^{Waf1}$ in Ras-expressing FVB cells are derived from genomic instability whereas in 16E5 cells this instability is diminished. Because cyclin D1 expression can be modulated by Ras either through MAPKs or PI3K pathways, and that 16E5 reduces the transforming activity of Ras (opposing activities), we speculate that one of the mechanisms through 16E5 could reach the cell life equilibrium is by modulating finally the levels of cyclin D1 mediated by MAPK/PI3K signaling pathways.

The GTPase Gpn1 is ubiquitinated by BRCA1

Griselda Peña-Gómez, Angélica Y. Robledo-Rivera, Roberto Sánchez-Olea and Mónica R. Calera.

Instituto de Física, Universidad Autónoma de San Luis Potosí. Manuel Nava #6, Zona Universitaria C.P.78290. San Luis Potosí, SLP. México. Tel: (444) 8262300 Ext 5717. Email: mcalera@ifisica.uaslp.mx

Gpn1 and Gpn3 are essential proteins that belong to the Gpn family of GTPases. They interact with each other and with the RNA polymerase II, whose nuclear targeting depends critically on both, Gpn1 and Gpn3. A TAP-MS global assay reported that Gpn1 and Gpn3 co-purified with the BRCT domains of breast cancer protein 1 (BRCA1). BRCA1 is a tumor suppressor hub protein involved in many cellular processes, including DNA repair, cell cycle checkpoint control, apoptosis and transcriptional regulation. BRCA1 forms an obligate heterodimer with BRCA1-associated RING domain 1 protein (BARD1); BRCA1 and BARD1 associate through their N-terminal RING domains. These domains also confer BRCA1/BARD1 an E3 ubiquitin-protein ligase activity towards H2AX, nucleophosmin and p53. However, not many ubiquitination substrates have been described for the E3 ligase activity of BRCA1/BARD1. Ubiquitin chains formed through different ubiquitin lysine residues signal differentially. Polyubiquitination through ubiquitin Lys48 serves as a degradation signal by the proteasome. In this work, we performed ubiquitination assays in HEK293T cells and showed that Gpn1-Flag is polyubiquitinated by the BRCA1/BARD1 protein complex. Increasing BRCA1/BARD1 and Ha-ubiquitin levels beyond a critical value led to a marked decrease in Gpn1-Flag protein levels, suggesting that BRCA1/BARD1 targets Gpn1-Flag for degradation by the proteasome. We are investigating the physiological consequences of Gpn1 polyubiquitination by BRCA1/BARD1.

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Determination of the indole-3-acetic acid site of action during somatic embryogenesis in *Coffea canephora*

Ana Odetth Quintana-Escobar y Víctor Manuel Loyola-Vargas

Centro de Investigación Científica de Yucatán, A.C. Calle 43 No. 130, Col. Chuburná de Hidalgo, C.P. 97200, Mérida, Yucatán, México. * Corresponding author: vmloyola@cicy.mx.

Somatic embryogenesis (SE) represents a powerful tool to study the morpho-physiological, biochemical and molecular processes that take place during the development of the embryo. The indole-3-acetic acid (IAA) has a fundamental role in the development processes of plants, so it has become the object of study to elucidate the mechanisms involved in its homeostasis. The analysis and visualization of the distribution of auxin is essential for the understanding of plant cell development, for which, synthetic promoters such as DR5v2, are used to respond to the action of IAA. The objective of our investigation is to determine the site of action of IAA during ES in *C. canephora*. We are using two systems for transformation with the vector: protoplasts and cell suspensions. 510,000 protoplasts mL-1 with a viability of 92% can be obtained from cell suspensions. In the case of cell suspensions, three fresh weight quantities (100, 150 and 300 mg) and three shaking times were evaluated with glass beads (20, 40 and 60 s) with a concentration of 16 µg of plasmid DR5v2. We have corroborated the transformation by confocal microscopy. The transformation in the treatments with 300 mg of cell suspensions and agitation time of 20 and 40 s was corroborated. Both the GFP signal and the tdTomato signal were observed, both included in the vector.

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Relevance of S-nitrosylation on cell death in reperfused hearts.

Nadia Giovanna Román-Anguiano, Francisco Correa-Segura, Agustina Cano- Martínez y Ana Cecilia Zazueta-Mendizábal. Instituto Nacional de Cardiología "Ignacio-Chávez". Juan Badiano No. 1, Col. Sección XVI, Delegación Tlalpan, C.P. 14080, D.F. Tel: 5573-2911 ext. 1465, mabbittzombiekill@gmail.com

After an ischemic event and as a consequence of reperfusion, cardiac tissue is further damaged in a phenomenon known as "reperfusion injury". Cell death by apoptosis, along with decrease in nitric oxide (•NO) content occurs during ischemia-reperfusion (I-R) and the application of low concentrations of this signaling molecule (10 nM to 1 µM) has been associated with cardioprotective effects [Loscalzo, 1995]. •NO regulates the function of the cardiovascular system through dependent and independent effects of cyclic guanosine monophosphate (cGMP). The independent cGMP pathway involves post-translational modification of proteins by S-nitrosylation. It has been described that •NO inhibits caspases and calpains by modifying critical cysteines of their catalytic sites by S-nitrosylation. So in this project we evaluated if the cardioprotective effects of •NO were related with S-nitrosylation of proteins involved in necrotic and apoptotic death pathways or if the main mechanism is associated with cGMP. We used two compounds that increase the levels of •NO by different mechanisms. 1) Prolame, an amino-estrogenic compound with antiplatelet and anticoagulant effects, that activates the endothelial nitric oxide synthase [Hernández-Reséndiz S, 2015] and, 2) S-Nitroso-N-acetylpenicillamine (SNAP), a synthetic donor that releases •NO, and that has been shown to decrease cell death after inducing hypoxia-reoxygenation in cell cultures [Rakhit RD, 2001].

We hypothesized that preserving the levels of •NO with Prolame or SNAP might decrease additional cell death generated by reperfusion by activating S-nitrosylation processes.

The administration of Prolame or SNAP confers cardioprotection to reperfused hearts, in association with decreased cell death by both necrotic and apoptotic pathways and increase in •NO content. We observed that the activity of proteases related to cell death processes decreases, which suggests that •NO may be modulating its activity through S-nitrosylation of cysteines in their catalytic site.

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Effect of Luteolin on the FADU cell line in the in the induction of apoptosis and cell migration.

Rosas Martínez Marisol, Gutiérrez Venegas Gloria, González Salguero Ricardo. Laboratorio de Bioquímica. División de Estudios de Posgrado e Investigación. Facultad de Odontología. UNAM. * Jefe de grupo : Laboratorio de bioquímica , División de Estudios de Posgrado de la Facultad de Odontología , Universidad Nacional Autónoma de México , Ciudad Universitaria , 04510 México, D.F., México. Phone (+52) (55) 5622-5554. Fax (+52) 5622-5554, email: gloria@fo.odonto.unam.mx

Abstract.

Cancer is characterized by the uncontrolled proliferation of cancer cells and by the repeated mitosis, which results in the invasion of other organs; metastasis. It is a disorder associated with papillomavirus (HPV) infection, tobacco or alcohol consumption and increases its presence in the younger population and in females due to changes in habits.

Apoptosis inhibition can prevent the physiological cell death that leads to the development and progression of malignant tumors. The inhibition of apoptosis can prevent the physiological cell death that leads to the development and progression of malignant tumors that affect the intrinsic pathway, regulated by proteins of the BCL-2 family; anti-apoptotic proteins (Bcl-2) and pro-apoptotic proteins (bax, bad, bid) and the extrineca pathway. Starting the activation of pro-caspase 8, this being the caspase initiator of apoptosis. Both pathways converge in caspase 3; responsible for nuclear apoptosis, having an effect on the cytoskeleton, cell cycle and signaling pathways, which constitute changes in apoptosis.

On the other hand, recent studies show that the use of some polyphenols of natural origin have shown clinical utility. Luteolin is a flavonoid belonging to this group of polyphenolic compounds; is derived from vegetables, fruits and tea; With anti-inflammatory, anti-cancer and anti-oxidant properties. Therefore, it could be a new alternative of treatment in patients with cancer. Hence the interest in the research entitled: Effect of Luteolin on the FADU cell line in the induction of apoptosis and cell migration, whose objective is to determine the effect of Luteolin on the FADU cell line in the induction of apoptosis and cell migration.

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Regulation and interaction of Retinoblastoma protein (Rb) by the Mdm2 oncoprotein in genotoxic stress conditions.

Adriana Berenice Rousset Román, Jesús Hernández Mongue, Vanesa Olivares Illana.
Laboratorio de interacciones moleculares y cáncer. Instituto de Física, UASLP.
vanesa@ifisica.uaslp.mx

The retinoblastoma protein (Rb) plays a critical role in cell cycle regulation and tumor suppression. Inactivation of Rb protein promotes continuous cell division leading to different human malignancies, including retinoblastoma. Has been reported that in retina cancer, the oncoprotein MDM2 is overexpressed. Mdm2 is a hub protein because its ability to interact with a large number of different partners among p53 is the best described. It has been also reported that Mdm2 interacts with the tumor suppressor Rb in normal conditions, under this circumstance Mdm2 is able to decrease the expression of Rb by two different mechanisms: ubiquitin-dependent and ubiquitin-independent degradation. However, under genotoxic stress conditions, MDM2 is ATM-dependent phosphorylated in Ser395 residue, while Rb is p38-dependent phosphorylated in Ser567. These phosphorylation events provoke conformational change that could change the partner of interactions or even the function of the proteins. Our interest is analyzing the post-translational modifications after genotoxic stress conditions and observes their role in the regulation of Rb by Mdm2. In the present study, we test the level of expression of Rb in presence of increasing concentrations of Mdm2 in normal and genotoxic stress conditions in different cells lines. We also studied the localization of the protein-protein or protein-RNA interaction by using ELISA co-immunoprecipitation and PLA assay.

High doses of IL-2 inhibit the proliferation induced by CD95 in cervical cancer cells

Itzel Salazar Valencia, Adriana Gutiérrez Hoya**, Octavio Zerecero Carreón, Arturo Valle Mendiola, Diego Morelos Laguna, Benny Weiss Steider and Isabel Soto Cruz
Laboratorio de Oncología Molecular, FES Zaragoza, Universidad Nacional Autónoma de México, Batalla 5 de mayo s/n Col. Ejército de Oriente, CP 09230, Ciudad de México, México.
***Investigador CONACYT. Tel: 56230796, adrianagh85@hotmail.com; itzelgpe@comunidad.unam.mx*

In Mexico, cervical cancer is the second cause of cancer death in women. An occurrence of 13,960 cases is estimated annually, with an incidence of 23.3 cases per 100,000 women. Cervical Cancer is associated with human papillomavirus (HPV) infection. HPV affects biochemical, molecular mechanisms and signaling pathways involved in the development of this neoplasia. Cervical tumour cells have acquired strategies to survive; one strategy is the use of growth factors such as IL-2. Our work group demonstrated the expression of the IL-2 receptor, as well as different responses of cervical cancer cells, evidencing that high doses of this cytokine induce cellular arrest in cervical cancer cells. The signals to induce cell death are altered in malignant cells, for example, the resistance of the CD95/CD95L pathway. Studies have shown that in different carcinomas the modulation of this pathway activates multiple signaling cascades like the MAP kinases, NF-KB, Src kinases or PI3K to induce survival, proliferation and autophagy of malignant cells. Our main interest was to evaluate if high doses of IL-2 affect the susceptibility of cervical cancer cells to activate the CD95 pathway. For this purpose we evaluated extracellular and intracellular expression of CD95 and the effect of an agonist antibody for CD95 on IL-2 treated cervical cancer cells. Cervical cancer cell lines HeLa (HPV18+), INBL (HPV18+), HER3+ (HPV18+), CaSki (HPV16+) and C33A (HPV-) were treated with 100 IU/m IL-2 for 48 hours and then incubated in the presence of agonist antibody for CD95 (DX2). Cells were incubated with specific antibodies (anti CD95 PE-Cy7 and anti CD95 APC) and CD95 was determined by flow cytometry. Cell proliferation was evaluated with violet crystal staining. Our results showed that HPV-positive cervical cancer cells express high levels of CD95 (90%) while HPV-negative C33A cell line express very low levels of CD95 (2-3%). Interestingly, all cell lines express CD95 intracellularly (>90%).The stimulation of the CD95 pathway in HeLa cells using low concentrations of the agonist induces cell proliferation. On the contrary, very high concentrations induce apoptosis. IL-2 treated HeLa cells incubated in the presence of the CD95 agonist decreased the proliferation induced by CD95 similar to the negative control. Concluding remarks: All cervical cancer cell lines express CD95. The stimulation of the CD95 pathway in HeLa cells can induce either proliferation or apoptosis depending on the agonist dose. IL-2 inhibits the proliferation induced by CD95. Our results suggest that cervical cancer cells use the CD95 pathway to promote proliferation and survival.

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Co-expression of PAK1 and its novel target CaMKIIγ in human breast cancer cell lines and breast tumor samples.

Hector Iván Saldivar-Cerón[1,2], Rosaura Hernandez-Rivas[1], Olga Villamar-Cruz[2], Luis Enrique Arias-Romero[2].
[1] Departamento de Biomedicina Molecular, Centro de Investigación y de Estudios Avanzados del IPN, Avenida Instituto Politécnico Nacional 2508, Zacatenco, Gustavo A. Madero, Ciudad de Mexico. C. P. 07360.
[2] Unidad de Biomedicina Molecular, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México. Av. de los Barrios Nº1, San Javier Tlalnepantla, Estado de México, Mexico. C. P. 54090. email: larias@unam.mx

ABSTRACT

p21-activated kinases (PAKs) are Cdc42/Rac–activated serine-threonine protein kinases that play an important role in physiological processes such as motility, survival, mitosis, and apoptosis. *PAK1* is amplified and/or overexpressed in about 25-30% of breast cancers, and understanding how it signals could have therapeutic implications. In order to identify the immediate direct targets of PAK1 in breast cancer cells, we performed a phospho-antibody array assay. Our results showed that several signaling molecules involved in breast cancer initiation and/or progression are hypo-phosphorilated in *PAK1* deficient breast cancer cells, including some well characterized PAK1 substrates and also some other proteins that could be regulated directly or indirectly by PAK1. One of these molecules is CaMKIIγ, a Calcium/Calmodulin-dependent Protein Kinase which recently has been associated to breast cancer initiation and progression. Here we show that CaMKIIγ is phosphorylated both *in silico* and *in vitro* by PAK1.

An *in silico* analysis using the GPS 3.0 software suggested that at least two threonine residues at the positions 277 and 287 are potential PAK1 phosphorylation sites. *In vitro* kinase assays using a GST tagged CaMKIIγ fragment corresponding to amino acids 212-317, showed that only threonine 277 is phosphorylated by recombinant PAK1. However, CaMKIIγ mutants lacking both threonine residues are still phosphorylated by PAK1, suggesting that additional PAK1 phosphorylation sites are still present in this CaMKIIγ fragment. In addition, co-localization and co-immunoprecipitation experiments showed that PAK1 interacts with CaMKIIγ in a cellular context, suggesting that a PAK1- CaMKIIγ cascade is important for the regulation of several cellular processes including cell cycle progression, migration, survival and proliferation. Finally, we observed that both proteins are overexpressed in highly aggressive breast cancer cells and in human breast tumor specimens.

Alteration of the chemosensory system in flies *Drosophila melanogaster* due to the deficiency of the transcriptional factor *escargot* (*esg*)

Iván Sanchez Díaz*#, Fernando Rosales Bravo+, Alba Yetlanezi Castillo Pineda*, René Hernández Vargas*, Verónica Narváez Padilla+, Enrique Reynaud Garza*
*Instituto de Biotecnología /UNAM Av. Universidad #2001, Col. Chamilpa C.P. 62210 Cuernavaca, Morelos.
+Centro de Investigación en Dinámica Celular, Av. Universidad #1001, Col. Chamilpa C.P. 62209 Cuernavaca, Morelos
idch@ibt.unam.mx

INTRODUCTION

The chemosensory systems in animals are vital for interaction with the environment. These systems are formed mainly by neurons establishing specific circuits for the control of complex behaviors. In flies of the species *Drosophila melanogaster*, these circuits control stereotypical behaviors such as: the attraction or repulsion towards food, the direction of flight towards a source of nutrients, the interaction with other flies or even courtship and mating. In previous works in the laboratory we showed that flies with loss of function of the *escargot* gene (*esg*), which is a transcriptional factor, presents alterations in the response to bitter substances such as nicotine. This line was called L4.

METHODOLOGY

Evaluation of gustatory and olfactory systems in flies by measuring the preference index (PI), the Courtship Index (CI) and the meeting latency (ML). Monitoring of the development of the proboscis, the main chemosensory organ in the fly, at different stages of development by immunofluorescence technic and expression of green fluorescent protein (GFP). *In vivo* evaluation of the taste neuron activity of the proboscis using the GCaMP5 system. In all the trials, flies deficient in the esg gene were compared with wild flies.

RESULTS

The flies with loss of function of the *esg* gene showed a high CI when they were evaluated with other males, that is, they courted other males. The IP showed that these flies perceive other males as if they were females and the ML indicates their copula success is very low. The immunofluorescence in larvae showed that there are alterations in the development of the labial imagales discs in flies deficient of the *esg* gene. These discs are the precursors of the proboscis in the adult fly. The complete absence of the *esg* gene causes lethality, however, low levels produce varied phenotypes, all of them associated with the loss of chemosensory structures. The evaluation of the neuronal activity of the proboscis showed that the response to caffeine and lobeline stimuli in flies deficient in the *esg* gene is diminished. Surprisingly, a response to the nicotine stimulus is observed, which is not observed in wild flies.

CONCLUSIONS

Our data showed that the development of the chemosensory system is affected in flies have a loss of function of the *esg* gene, in addition, the functionality of the neurons as well as the receptors present in the proboscis are directly altered by the loss of function of this gene, which indicates a primordial role in the development and functioning of the peripheral nervous system (SNP).

The peroxisome import receptor dislocation complex restrains peroxisome removal in *Podosopora anserine*

Fernando Suaste-Olmos, Harumi Takano-Rojas, Claudia Zirión-Martínez, Leonardo Peraza-Reyes
Instituto de Fisiología Celular, Universidad Nacional Autónoma de México. Circuito Exterior s/n. Ciudad Universitaria, Coyoacán, 04510 Mexico City, Mexico. Tel. (55) 5622-5628.
fsuaste@ifc.unam.mx, lperaza@ifc.unam.mx

Peroxisomes are versatile organelles that are involved in multiple tasks, been the most conserved the metabolism of lipids and of reactive oxygen species. In our group we study the function and dynamics of peroxisomes through sexual development of the filamentous fungus *Podospora anserina*. Peroxisomes import proteins from the cytosol into its lumen through a complex machinery inserted in their membranes, which is composed of two principal components: the import machinery (the importomer) and the peroxisome receptor export complex (the exportomer). The importomer facilitates the insertion of cargo-loaded import receptors into the peroxisome membrane to promote the assembly of a peroxisome membrane selective pore through which proteins are translocated into the organelle. The exportomer then facilitates the dislocation of the translocation pore from the membrane promoting the recycling of the import receptors and the translocation of their cargos. The activity of the exportomer relies on two protein subcomplexes: the ubiquitination complex, which primes the receptors for recycling, and the receptor dislocation complex, which extracts the ubiquitinated receptors from the membrane. In this research we show that, in addition to its participation in peroxisome protein import, the receptor dislocation complex is also required to restrain peroxisome removal in this fungus. We show that elimination of either dislocation complex protein results in peroxisome loss under specific physiological conditions, and this process is accompanied by relocalization of peroxisome membrane proteins into the vacuolar lumen, consistent with a vacuolar degradation of peroxisomes. In addition, we show that this phenotype is not observed upon elimination of other ubiquitination complex or importomer proteins. Our results disclose a critical role for the peroxisome receptor dislocation complex in modulating peroxisome abundance in *P. anserina*. This research was supported by PAPIIT grant IA203317 from DGAPA, UNAM.

Regulation of the BarA/UvrY two component signaling system.

Silvia Fernanda Urias Contreras, Katherine Ali de Jesús Valencia Sánchez, Archana Pannuri, Tony Romeo, Adrián Fernando Álvarez, and Dimitris Georgellis
Instituto de fisiología Celular; Universidad Nacional Autónoma de México, Circuito Exterior s/n Ciudad Universitaria, Coyoacán, 04510 México D.F., Department of Microbiology and Cell Science, University of Florida Tel. 56225738. surias@email.ifc.unam.mx

The BarA/UvrY two-component system mediates adaptive responses of *Escherichia coli* to changes in growth stage. At late exponential growth phase, the BarA sensor kinase senses and responds to acetate, leading to its auto-phosphorylation and transphosphorylation of UvrY, which activates transcription of the CsrB and CsrC noncoding RNAs. CsrB and CsrC, in turn, sequester the RNA binding protein CsrA, which posttranscriptionally regulates translation and/or stability of its target mRNAs, antagonizing its regulatory functions.

Curiously, CsrA appears to be required, although indirectly, for the activation of BarA. Therefore, it was suggested that, in addition to acetate, other factors, whose expression depends on CsrA, might be involved in the control of BarA activity.

Here, we provide results of experiments aiming at identifying possible intermediate proteins involved in the activation mechanism of the BarA/UvrY-CsrA circuitry.

The implications of our findings on the regulation of the BarA kinase activity will be discussed.

Novel unique ligands of SmicRACK1 protein from the cnidarian symbiont *Symbiodinium microadriaticum* ssp. *microadriaticum*.

Tania T. Islas-Flores, Pijush Mallick and Marco A. Villanueva.

Instituto de Ciencias del Mar y Limnología, Unidad Académica de Sistemas Arrecifales, Universidad Nacional Autónoma de México-UNAM, Prol. Avenida Niños Héroes S/N, C.P. 77580, Puerto Morelos, Quintana Roo, México. Email: marco@cmarl.unam.mx

The Receptor for the Activated C Kinase 1 (RACK1) is a multifaceted protein ubiquitously expressed in eukaryotes. This protein has gained interest because it is involved in multiple signaling pathways and cellular processes that include translation, photosynthesis, cell wall biogenesis, stress and defense response, growth, development, and hormonal control. This ability is due to its beta-propeller structure formed by seven WD-40 repeats, which allows several ligands to associate concomitantly, thus forming multiprotein complexes. By functioning as an interaction hub for several proteins, RACK1 can be used as a bait to identify its ligands and characterize signaling pathways. In the last decades, bleaching has become one of the main causes of coral death and coral reef destruction; it consists in the loss of the functional symbiosis between the coral and its symbiont *Symbiodinium*. Hence, we focused on identifying signaling pathways that may underlie the cnidarian-*Symbiodinium* symbiosis in order to understand the biochemical and molecular mechanisms of coral bleaching. To identify potential signaling pathways involved in symbiosis in *Symbiodinium microadriaticum*, we generated a cDNA library to screen ligands of the *S. microadriaticum* RACK1 homolog SmicRACK1, by the Yeast Two-Hybrid System. This library was readily obtained with the DINO-SL oligo, which proved to yield complete transcripts and provide good representation. The screening with SmicRACK1 as "bait" allowed us to identify 7 ligands, 4 of which were identified as a Hemerythrin-like protein, E3 ubiquitin-protein ligase SIS3, Calcium-binding protein NCS-1, and KDEL motif-containing protein 1. The three remaining ligands resulted to be unique to *Symbiodinium* since they did not present homologs from other organisms. These new ligands will shed light into the function of SmicRACK1 in *Symbiodinium*, including its interactions and symbiosis with the cnidarian host.

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Effect of IL-2 on the secretion of lactate and the NADH/NAD+ ratio in cervical carcinoma cell line SiHa

Arturo Valle-Mendiola, María del Carmen Lagunas-Cruz, Benny Weiss-Steider, Isabel Soto-Cruz. Laboratorio de Oncología Molecular L9PB, Unidad Multidisciplinaria de Investigación Experimental Zaragoza, Facultad de Estudios Superiores Zaragoza, UNAM. Avenida 5 de mayo, esquina Fuerte de Loreto S/N, colonia ejército de oriente, Iztapalapa. Tel. 52 55 56230796. E-mail: arturo.valle@unam.mx; sotocruz@unam.mx

Cervical Cancer is the second cause of death in women worldwide, in Mexico, the highest incidence in gynaecological tumours is breast cancer followed by cervical cancer. The IL-2R has been found to be expressed on non-haematopoietic cells, especially in several types of solid tumours. However, the function of this receptor on malignant cells has not been defined. We documented the expression of the IL-2R and the production of IL-2 in cervical cancer cells that induces its proliferation. A relevant characteristic of many types of cancer is the ability to reprogram energy metabolism to fuel cell growth and division. The increased uptake of glucose and its conversion into lactate is one hallmark of cancer cells, usually, in these cells, the excess of lactate is secreted to modify tumour microenvironment promoting tumour cell invasion and metastasis formation. To analyse the effect of IL-2 on lactate production and on the NADH/NAD+ redox state we treated cervical carcinoma cell lines with high (100UI/mL) or low (10UI/mL) doses of IL-2. The cell line SiHa was treated with IL-2 and the metabolites were measured by using the Lactate Assay Kit or the NADH/NAD+ quantitation kit. Our results show that the treatment with low doses of IL-2 induces an increase in the secretion of lactate, nevertheless, with high doses the production of lactate decreases. The NADH/NAD+ ratio was consistent with a high metabolic flux, typical of transformed cells, where a high NADH/NAD+ redox state is capable of sustaining aerobic glycolysis. The role of IL-2 in the metabolic switch of cancer cells is poorly studied. Here we show that IL-2 induces a change in lactate secretion and the redox state of the cell which is dose-dependent. These results suggest that IL-2 regulates glucose conversion into lactate to increase its concentration for intracellular acidification giving cervical cancer cells an advantage to proliferate and to migrate.

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The adenosine derivative IFC305 inhibits the fibrotic phenotype of cultured activated hepatic stellate cells.

Nora Gabriela Velasco- Loyden, Enrique Chávez Jiménez, Jesús Rafael Rodríguez- Aguilera, María Guadalupe Lozano Rosas and Victoria Chagoya de Sánchez*. Instituto de Fisiología Celular, UNAM. Circuito Exterior s/n Ciudad Universitaria, Coyoacán, 04510 México, CDMX, +52 55 56225614, * vchagoya@ifc.unam.mx.

Liver hepatic stellate cells (HSC) are quiescent cells with large vitamin A lipid droplets and an adipogenic phenotype. During the development of liver fibrosis in chronic liver diseases quiescent HSC transdifferentiated to myofibroblast acquiring a fibrotic phenotype characterized by increased proliferation and excessive synthesis of extracellular matrix (ECM) proteins, especially type I collagen. We have previously shown that the adenosine derivative IFC305 reverses pre-established CCl_4-induced cirrhosis in rats (Perez-Carreon et al. 2010) and this effect involves epigenetic modifications in the whole liver (Rodríguez- Aguilera JR, 2018). We have also demonstrated that this compound suppresses the *in vitro* transdifferentiation of rat quiescent HSC by inhibiting collagen α1(I) expression, and up-regulating $PPAR_Y$, MMP13, and Smad7 expression (Velasco-Loyden et al. 2010).

The aim of this work was to characterize the effect of IFC305 on rat liver HSC derived myofibroblasts. HSC were isolated from normal male Wistar rats and cultivated for 7 days to activate them, then, they were incubated in the presence of IFC305 for 48 hours or in a continuous treatment for the following 7 days. We also included the BSC10 cells in our studies, a HSC rat activated cell line. We found that IFC305 reduced the proliferation of activated HSC in a dose dependent manner. In addition, IFC305 blocked the expression of the pro-fibrogenic markers collagen α1(I), TGF-β1, up-regulated the expression of the anti-fibrogenic gene PPAR and reestablished some adipogenic characteristics. Considering the increased evidence that epigenetic regulation have an impact on HSC activation and that the IFC305 compound is able to modulate epigenetic changes, we are also exploring this molecular mechanism as part of the IFC305 anti-fibrotic actions.

PARTICIPATION OF AQP8 IN THE MODULATION OF METABOLIC PATHWAYS ACTIVATED BY ADRENALINE IN HEPATOCYTES.

María Magdalena Vilchis Landeros[1], Raquel Guinzberg Perrusquia[1], Alma Andrade Cedillo[1], Guillermo Morales Hernandez[1], Antonio Díaz-Cruz A[2] y Enrique Piña Garza[1].
[1] Departamento de Bioquímica, Facultad de Medicina, [2] Departamento de Nutrición Animal y Bioquímica, Facultad de Medicina Veterinaria y Zootecnia. Universidad Nacional Autónoma de México (UNAM), Ciudad de México, 04510, México. Tel: 52 55 56232510. vilchisl@unam.mx

Currently, H_2O_2 has been consolidated as the central metabolite in the signaling of redox systems, it functions as a second messenger that rivals cAMP and Ca^{2+} in its capacity for signaling and regulation. Adrenaline is one of the hormones that modulate the availability of H_2O_2 in various cells. Recently, our group reported that the stimulation of alpha1 adrenergic receptors in isolated hepatocytes activates the Nox2 of the membrane, which increases the formation of H_2O_2. This H_2O_2 prevents the elevation of gluconeogenesis and ureagenesis mediated by stimulation of the beta-adrenergic receptors present in cells and stimulates these metabolic pathways mediated by alpha1-adrenergic receptors. Aquaporins (AQP) are membrane proteins responsible for carrying molecules through the cell membrane; 3 and 8 specifically transport H_2O_2. This work shows that H_2O_2 must necessarily penetrate the hepatocyte to exert its effect, and does so exclusively through aquaporin 8, whose presence we have demonstrated in the rat hepatocyte by means of RT-PCR and western blot. The general scheme is enriched and complicated by the fact that in these hepatocytes isolated and incubated in the presence of nanomolar concentrations of H_2O_2 or the H_2O_2 generated for the action of adrenaline through the alpha1 receptors, they promote the exit of calcium from intracellular pools to the cytosol, and this effect is inhibited by the presence of anti-AQP8 antibodies. Our experiments have shown that aquaporin 8 participates in the actions of adrenaline signaling in hepatocytes and could place H_2O_2 at the precise site to oxidize to the next protein in its signaling cascade.

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Identification of a nuclear export signal in the GTPase Npa3

Tania A. Félix-Pérez, Martín A. Mora-García, Roberto Sánchez-Olea and Mónica R. Calera.
Instituto de Física, Universidad Autónoma de San Luis Potosí, Manuel Nava #6, Zona Universitaria, C.P. 78290, San Luis Potosí, SLP, México. Tel: (444) 8262300 Ext 5717. Email: mcalera@ifisica.uaslp.mx

The GTPase Npa3 is an essential protein in the yeast *Saccharomyces cerevisiae*, possibly due to its important role in the nuclear accumulation of RNA polymerase II, although the precise underlying molecular mechanism is still being investigated. Recently, our group demonstrated that the C-terminal tail of Npa3 is dispensable for cell proliferation. However, removal of this region resulted in the retention of the Npa3ΔC-279 protein in the cell nucleus. It has been shown that human Gpn1 has a very effective nuclear export signal (NES) at the C-terminal tail, which is recognized by the exportin Crm1. Interestingly, Npa3 presents a conserved sequence in the same region. In this work, we investigated if Npa3 NES is functional in this protein. To achieve this goal, molecular constructs of different versions of Npa3ΔC fused to GFP containing this NES were generated. The results showed that wild-type Npa3-GFP was located in the cytoplasm and Npa3-279-GFP was concentrated in the cell nucleus, as expected. Npa3ΔC-295-GFP had a nuclear and cytoplasmic distribution, and Npa3ΔC-310-GFP was excluded from the cell nucleus. To evaluate the importance of the NES sequence in the context of the full-length protein (Npa3-385), the subcellular distribution of Npa3-385-NESmut-GFP, a mutant in two hydrophobic amino acids (L293A/L295A) conserved in the NES consensus sequence, was determined. We found that Npa3-385-NESmut-GFP was strongly concentrated in the cell nucleus of the yeast *S. cerevisiae*. The results obtained in the present work indicate that Npa3 is constantly mobilized between the cell nucleus and the cytoplasm, and that the mainly cytoplasmic localization of Npa3 is due to the high effectiveness of the NES present at the C-terminal tail of Npa3. This study is the first report that shows the functionality of the nuclear export signal in Npa3.
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Evaluation of the energetic metabolism and redox state of senescent breast epithelial MCF-10 cell line

Angélica Alejandra Aquino-Cruz[&*], Verónica Salas Venegas[&*] , Sharik Hernández Rizo[&*], Rafael Toledo Pérez
[&*], Rebeca López-Marure[#], Mina Königsberg-Fainstein[&], Norma Edith López-DíazGuerrero[&***]
·#Departamento de Fisiología. Instituto Nacional de Cardiología "Ignacio Chávez".
*Posgrado en Biología Experimental, UAM Iztapalapa.
[&]Universidad Autónoma Metropolitana Iztapalapa, Av. San Rafael Atlixco 186, Leyes de Reforma 1ra Sección,09340 Ciudad de México, CDMX. Tel. 5524937625 *** norm@xanum.uam.mx

Introduction. Cellular senescence refers to the essentially irreversible arrest of cell proliferation that occurs when cells experience some stress that induces DNA damage. As in the case of oxidative stress, one of the models of induction of senescence is premature senescence induced by stress (SIPS))where DNA damage is induced. Most studies on senescent cells have been performed on fibroblasts; however, it is essential to know the behavior of senescent cells of other cell types and how they contribute to different pathologies. A large number of neoplasms have an epithelial origin,and senescent epithelial cells have been found to be involved in the maintenance and progression of tumors. Currently, little is known about the parameters of these cells such as energy metabolism and the redox state present. An important and little studied is the controversial aspect of the knowledge of energy metabolism of senescent cells, since some authors mention that it is predominantly glycolytic and others that it goes more towards oxidative phosphorylation. Clarifying the type of metabolism that predominates in the senescent cells could be an important key to understand its role in cancer, and in a future like a therapeutic target to eliminate these cells in a pharmacologically way.

Methodology.
The MCF10A cell line was induced to senescence by SIPS to determine the senescent parameters and the glycolytic activity and oxidative phosphorylation. The seahorse extracellular flow analyzer was used. For the redox state, the GSH / GSSG coefficient was determined by HPLC. The intracellular concentration of ROS was measured with a Cellular Reactive Oxygen Species Detection Assay Kit (Deep Red Fluorescence). **Results.** Oxygen consumption and glycolytic activity in senescent cells were lower in senescent cells. Changes were observed in the GSH /GSSG coefficient with respect to the control, as well as the intracellular concentration of ROS.

Conclusions. We observed changes in both the metabolism and the redox state of senescent breast epithelial MCF-10 cell line by SIPS, compared to the control in different days of culture.

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Changes in expression in the EhTRF-like proteins of *Entamoeba histolytica* during oxidative stress

Karen Anell Becerril Puente[1], David Hernández-Álvarez[1] , Rosa Elena Cárdenas-Guerra[1], Elizabeth J. Castañeda-Ortiz[1], Lilia López-Canovas[1], Abigail Betanzos[2,3], Jesús Valdes Flores[4], Esther Orozco[2] and Elisa Azuara-Liceaga[1*]
[1]Posgrado en Ciencias Genómicas, Universidad Autónoma de la Ciudad de México. Ciudad de México. [2]Departamento de Infectómica y Patogénesis Molecular, Cinvestav-IPN. Ciudad de México. [3]CONACYT, Ciudad de México. [4]Departamento de Bioquímica, Cinvestav-IPN, Ciudad de México.
*San Lorenzo 290, Col del Valle Sur, 03100 Ciudad de México, Teléfono: 11070280 Ext. 15302. Correo electrónico: elisa.azuara@uacm.edu.mx

Abstract
Telomeres are specialized structures at the end of chromosomes essential for maintaining genome stability and cell viability. Telomeres are nucleoprotein complexes preventing chromosome termini from being recognized as broken DNA ends. Human telomeres contain two TTAGGG repeat binding factors, TRF1 and TRF2. TRF1 negatively regulates telomere elongation, while TRF2 protects the chromosome ends by inhibiting end-to-end fusions. TRF1 and TRF2 are essential in the response to DNA-damaging agents such as reactive oxygen species (ROS) and may play a role in telomere length regulation. In response to DNA-damaging agents TRF1 and TRF2 changes its expression modulating its presence at the telomeres. *Entamoeba histolytica* contains three genes encoding for EhTRF-like proteins with significant sequence similarity with TRF1 and TRF2. These proteins were dubbed EhTRF-like I, II, and III. EhTRF-like I and II shares similarity with TRF 1, while EhTRF-like III shares similarity with TRF 2. The objective of this work was to determine changes in the expression of EhTRF-like I and III and analyzed their nuclear localization due to oxidative stress. We used qRT-PCR, western blot and immunofluorescence to analyzed the effect of DNA damage induced by H_2O_2 (0.4 and 2.5 Mm) and UV treatments (150 J/m^2). Our results showed that *ehtrf-like* genes overexpressed immediately after treatment when sub lethal H_2O_2 concentrations and are repressed under high peroxide concentrations and UV treatments. In basal conditions, TRF-like I and III proteins are localized into the nuclei near to the nuclear membrane co-localizing with lamin B1 and in *foci-like* structures. However, after both treatments TRF-like proteins are diminished at the nucleus. We observed the highest co-localization inside the nucleus at 6 hours of recovery from both treatments. These results provide the first evidence that EhTRF-like proteins might have a role during damage to DNA from the instability caused by oxidative stress in this parasite.

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Antioxidant enzymatic system of the antarctic yeast *Rhodotorula mucilaginosa* M94C9; a biochemical and bioinformatic – comparative study

Jorge Brito Sánchez[1], Ana Paulina Mendoza von der Borch[1], Marcelo Baeza Cancino[2], Viviana Escobar Sánchez[1], Claudia Segal Kischinevzky[1] y Luisa Alvarina Alba Lois[1]
[1]Facultad de Ciencias, UNAM. Laboratorio de Biología Molecular y Genómica. Facultad de Ciencias. Circuito de la investigación científica. Ciudad Universitaria. Delegación Coyoacán, CDMX. C.P. 04510. Contacto: 5526898256, jorge.brito@ciencias.unam.mx
[2]Departamento de Ciencias Ecológicas Facultad de Ciencias Universidad de Chile

Oxidative stress (OS) is a physiological state involved in aging and different pathological conditions: carcinogenesis, atherosclerosis, diabetes, neurodegenerative disorders, etc. The main responsible for this type of stress are reactive oxygen species (ROS) and the cellular antioxidant system and it is generated due to an imbalance between both components favoring of ROS. The major ROS of physiological importance are superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (• OH). Cell damage due to ROS excess is prevented by the antioxidant system, a set of enzymatic and non-enzymatic mechanisms whose ultimate goal is to neutralize the reactivity of these molecules. Superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) are the main enzymes of the antioxidant system, their function is thought to be necessary for life in all cells that live in presence of oxygen.

A particular combination of some conditions that favor OS occurs in Antarctica, the coldest and driest continent on planet Earth: high incidence of UV radiation, low nutrient availability due to freezing temperatures and freeze-thaw cycles common to antarctic soils. From one expedition to Antarctica Marcelo Baeza's workgroup isolated, identified and characterized 16 strains of cold adapted yeasts, among them *Rhodotorula mucilaginosa* M94C9.

Protein studies that evaluate the amino acids percentage show that cold adapted methanogenic archaeobacteria have a higher content of non – charged polar amino acids and lower content of hydrophobic amino acids, while in cold adapted γ - proteobacteria it has been observed there is a higher content of Asp, Asn , Ile, Lys and Ser and lower content of Ala, Pro, Arg, Gly and Leu.

In the present work we determinate the SOD, catalase and GPx activities in different growth phases of *R. mucilaginosa* M94C9 growing at 28 ºC and 4 ºC.

On the other hand, we want to establish the amino acids proportion that characterizes proteins of cold-adapted yeasts (psychrophilic and psychrotolerant) as compared to proteins of mesophilic fungi and to determine whether the GPx and SOD sequences of an antarctic strain of *R. mucilaginosa* share characteristics with proteins of cold adapted yeasts.

So far results show that catalase has more activity in the stationary phase of growth than in the exponential phase, in contrast, SOD is more active in the exponential phase than in the stationary phase.

Combination of single doses of Cytarabine and Ferric Carboxymaltose (Fe^{+3}) increases oxidative damage and alter redox balance in rat brain

David Calderón Guzmán,[1] Maribel Ortiz Herrera,[2] Hugo Juárez Olguín,[3] Francisca Trujillo Jiménez,[3] Miroslava Lindoro Silva,[3] Ernestina Hernández García.[3] Laboratories of Neurosciences,[1] Experimental Bacteriology,[2] Pharmacology[3]
Medicine Experimental Division. National Institute of Pediatrics.
phone: (52)10840900. Email:solodavid2001@yahoo.com.mx

Abstract
Anemia still represents a major complication of treatment in cancer patients aggravating physical impairment, and recent studies suggest an association between iron metabolism and antioxidative system in patients with acute myeloid leukemia during cytarabine chemotherapy. The oxidative damage of this anticancer agents and Ferric Carboxymaltose, FC (Fe^{+3}) remain unclear. The purpose of this study was to measure the effect of cytarabine on some select oxidative biomarkers in the brain regions of rats treated with FC (Fe^{+3}). Twenty male Wistar rats were randomly distributed in 4 groups, which were given single dose of cytarabine (25mg/rat) and FC (50mg/rat) intraperitoneal. The treatment was given in the following way: control group 1 NaCl 0.9%; Cytarabine (Cyt), group 2; FC, group 3; Cytarabine + FC, group 4. The animals were sacrificed by decapitation and their brains were dissected in cortex, hemispheres, and pool of cerebellum/medulla oblongata. Blood samples were obtained to assess the levels of haemoglobin. Each region was used to assays Lipoperoxidation, H_2O_2, Ca^{+2}, Mg^{+2} ATPase and Glutathione (GSH), using fluorescence methods. Hemoglobin levels increased in the group treated with Cyt + FC. H_2O_2 levels decreased in cortex regions, and increased in hemispheres regions in the group that received Cyt + FC, and was increase in cerebellum/medulla oblongata regions in the group that received FC. Lipid peroxidation was increase in the groups that received FC or Cyt in cortex regions, and this biomarker increased in all the experimental groups. GSH levels decreased in groups treated with Cyt + FC in cortex regions. ATPase activity increased in cortex regions of the groups treated with FC, and in hemispheres this enzyme activity increased with the combination of Cyt + FC. The results suggest that the increase of oxidative biomarkers during cytarabine and FC administration resulted in brain injury and could potential the oxidative stress in patients.

Expression of peroxyrredoxin 3 in squamous intraepithelial lesions and cervical cancer

Ruth Yareli Calvario Pino[1], Daniel Hernández Sotelo[1], Oscar del Moral Hernández[1], Luz del Carmen Alarcón Romero[1], Yaneth Castro Coronel[1].

[1]Laboratorio de investigación en Citopatología e Histoquímica. Facultad de Ciencias Químico Biológicas. Universidad Autónoma de Guerrero. CP 39090. Av. Lázaro Cárdenas, S/N. Ciudad Universitaria. Chilpancingo, Gro. Tel/Fax (747)4710901.
e-mail: yanethcc@yahoo.com.mx

Introduction: Oxidative stress (EO) is the overproduction of reactive oxygen species (ROS) and / or loss of the antioxidant defense system, a process to which cells are exposed due to different intrinsic and extrinsic factors (1), This condition, combined with human papilloma virus (HPV) infection, results in a feasible condition for the appearance of a squamous intraepithelial lesion (LEI) and promotes a favorable environment for its progression (2). Recently EO has been studied due to the high levels that have been found in different types of cancer, peroxirredoxin 3 (PRX 3) is an antioxidant protein that acts under EO conditions because it can modulate the overproduction of ROS, it has been studied as a possible biomarker for the detection of oxidative damage in the progression of LEI and cervical cancer (CaCu) due to overexpression of this protein in neoplastic processes (3) (4). **Objective:** To evaluate the expression of PRX 3 in LEI and CaCu. **Methodology:** Samples of 75 women were analyzed: 15 cytologies without LEI and without HPV, 15 without LEI with HPV, 15 with LEIBG, 15 LEIAG and 15 of cervical cancer, expression of PRX 3 was determined by immunocytochemistry and quantified by software Image Pro-Plus. **Results and discussion:** Five types of HPV-AR were identified by the INNO-LiPA HPV Genotyping method, being the most frequent HPV-16 (24.9%), however, 64.82% of the population suffers multiple infection (MI) due to HPV. , where significant differences were found ($p < 0.001$) between the degree of the lesion and the type of HPV. The quantification of the immunocytochemistry showed a gradual increase of PRX 3, in the low-grade LEI groups (LEIBG), high-grade LEI (LEIAG) and CaCu, showed statistical significance ($p < 0.001$). **Conclusion:** The expression of PRX 3 increases gradually according to the degree of cytological lesion and according to the presence of HPV, so the evaluation of this protein could be complementary in the timely detection of CaCu.

Debaryomyces hansenii catalase T gene overexpression on an acatalasemic strain of *Saccharomyces cerevisiae*

Román Alfonso Castillo Díaz, Viviana Escobar Sánchez, Claudia Andrea Segal Kischinevzky
Laboratorio de Biología Molecular y Genómica, Facultad de Ciencias, UNAM
Av. Universidad 3000, Circuito Exterior S/N, Delegación Coyoacán, C.P. 04510, Cd.Mx.
castillodiazroman@gmail.com

Aging is a multifactorial process. One of the principal factors is the accumulation of the free radicals, in particular, the reactive oxygen species (ROS). The oxygen, essential for the synthesis of ATP for aerobic organisms, is also responsible for damaging the biomolecules and for aging, at least partially. When reactive oxygen species overcome the antioxidant capabilities of the cell, the oxidative stress emerges.

Due to the oxygen damage, organisms can detect, determine the concentration, mitigate and repair the damages caused by ROS. The cells keep the normal parameters of ROS through various mechanisms, including some antioxidant enzymes like catalase, which carries out the decomposition of hydrogen peroxide to oxygen and water, this enzyme also works as a key in the control of ROS, because hydrogen peroxide can, under certain conditions, give rise reactive species such as radical hydroxyl, causing irreversible damage to cells.

Debaryomyces hansenii is an unconventional yeast with great potential for biotechnology, one of its qualities is the high tolerance to hydrogen peroxide. In previous work, carried out in our laboratory, it was determined that catalase activity is greater than in *Saccharomyces cerevisiae*, and that this may explain this tolerance.

Because of the high tolerance to hydrogen peroxide of *D. hansenii* associated to the elevated catalase activity, we cloned the catalase T gene into the plasmid pYES2 under the control of GAL1 promoter, this construct was transformed into an acatalasemic strain of *S. cerevisiae*. Viability curves, catalase activity assay, growth curves, and viability assays after hydrogen peroxide shock in different media and growth phases where performed.

We found out that the over-expression of *D. hansenii* catalase T decreased the chronological life span of *S. cerevisiae*, at least in the conditions in which the viability curves were performed. By the other hand, the growth rate and the biomass reached in both, rich and minimal medium, with or without induction, is not affected. The tolerance to a hydrogen peroxide shock successfully increased, as well as the catalase-specific activity in the presence of GAL1 inductor.

Impact of Resveratrol on the temporal lobe of Wistar rats treated during the aging process

Iván Cesar Arteaga (Estudiante de Licenciatura)[1]; Eduardo Miguel Brambila Colombres [3,4]; Lourdes Millán Pérez Peña[4,5]; Addi Rhode Navarro Cruz[1]; Patricia Aguilar Alonso [1,4];

[1] Departamento Bioquímica-Alimentos, Facultad de Ciencias Químicas. BUAP; [2] Departamento De Análisis Clínicos, Facultad de Ciencias Químicas. BUAP; [3] Posgrado en Ciencias Químicas. BUAP; Centro de Química, BUAP. agapmx@yahoo.com.mx.

Aging is a biological process that produce in all living beings, the passage of time affects brain function mediated by reactive oxygen species (ROS) that attack mainly the polyunsaturated fatty acids that make up the lipid bilayer, leading to cell death causing long term from learning difficulty to senile dementia; so the damage products are considered a study tool. Molecules capable of protecting cells by oxidative damage have been studied by diverse authors, one of them is the Resveratrol, our work has as aim know the effect of the resveratrol on the temporal lobe of aging rats. The results show that at 2, 4, 6 and 8 months of treatment with resveratrol, the concentrations of nitric oxide and lipoperoxidation products decrease, as well as the increase of the enzymatic activity (SOD, CAT), it study can be concluded that Resveratrol acts as a good antioxidant decreasing oxidative stress during the aging.

Variants in SOD2, CAT, GPX1 and GPX7 genes as markers of oxidative stress associated with obesity in children from Mexico City.

Cota-Magaña Ana Isabel[1,2,3], Díaz-Flores Margarita[3], Valladares-Salgado Adán[3], López-DíazGuerrero Norma Edith[2], *Cruz-López Miguel[3].
[1]Doctorado en Ciencias Biológicas y de la Salud, UAM Iztapalapa. [2]Lab. Bioenergética y Envejecimiento Celular, UAM Iztapalapa. [3]U. de Investigación Médica en Bioquímica, UMAE Especialidades "Bernardo Sepúlveda", CMN S. XXI, IMSS. *Responsable: Acad. Dr. Miguel Cruz Jefe, Titular D, SNI III Av. Cuauhtémoc 330, Col. Doctores C.P. 06720, Tel +(55) 5761-2358, Fax +(55) 5627-6914.

Background: Obesity is recognized as a public health problem in Mexico and the world. This condition is associated with a state of oxidative stress and low-grade inflammation. It has been reported that obese children have alterations in enzymatic activities, mainly of SOD and GPX, as well as high oxidant stress markers (Malondialdehyde and carbonylated proteins). Interestingly, there is evidence indicating that some genetic variants in antioxidant enzymes are associated with childhood obesity; however, the evidence on this is still controversial and scarce.

Objective: To determine the association of genetic polymorphisms in SOD2, CAT, GPX1 and GPX7 genes with changes in their enzymatic activity, and their relationship with the generation of oxidative stress in childhood obesity.

Methods: We analyzed 200 boys and girls between 6 and 12 years of age from the Unidad Deportiva Morelos del IMSS; the children were categorized by normal weight and obesity, according to the percentiles of the CDC (obesity ≥90th percentile). The complete biochemical profile was determined (LDLC, CHDL, Triglycerides, cholesterol, glucose). With the DNA samples, the polymorphisms of SOD2 (rs4880), GPX1 (rs1050450), GPX7 (rs538337) and CAT (rs100117) were analyzed by PCR. The antioxidant activities of the enzymes SOD, CAT, GPX, and oxidized proteins were measured in lysates of erythrocytes, and lipoperoxidation in plasma. In addition to measured insulin to calculate the HOMA-IR and the insulin resistance.

Results: Obese children presented high levels of LDL-C, TGC, and decreased CHDL, HOMA-IR and insulin resistance in 16% of children. An increase in the activity of the enzymes SOD, CAT, and GPX, and an increase of carbonylated proteins were observed. Genetic variants were not associated with obesity. However, carriers of the rs4880 genetic variant of the SOD2 gene show correlation with an increase in enzyme activity in children with obesity.

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Determination of antioxidant capacity in the organic waste of *Tenebrio molitor* larvae

Dinora Guadalupe Díaz-Parra, Vania Montserrat Salazar-Camarillo, José de Jesús Luna-Díaz, Ana Cristina Ramírez-Anguiano, Sandra Fabiola Velasco-Ramírez *.
Chemistry Department, Centro Universitario de Ciencias Exactas e Ingenierías (CUCEI), Universidad de Guadalajara. Blvd Gral. Marcelino Barragán Zip code 1421, Guadalajara, Jalisco.
* Blvd. Marcelino García Barragán #1421, esq. Calzada Olímpica, Zip Code 44430, Guadalajara, Jalisco, México. Phone (33) 13785900 ext. 27517. E-mail: sfqfb30@gmail.com

Knowlegde area #4 Reactive Oxygen Species

ABSTRACT

Entomophagy is a food habit known as the consumption of insects and/or their by-products, which presents studies and scientific evidence that shows that insects represent a protein source more sustainable than conventional ones (cattle). However, they are not organoleptically acceptable in many cultures, which makes the consumption of them not profitable practice.

The organic waste of *Tenebrio molitor* is a by-product produced by the larvae of this insect, which is nutrimentally balanced, organoleptically acceptable and possesses functional activity.

Because the investigations that exist in functional foods are only oriented to conventional foods, in this project we focus on the study and analysis of unconventional foods. In this case from the organic waste of the *T. molitor* insect we analyzed the antioxidant activity by various chromogenic compounds (ABTS, DMPD and FRAP) which are used to determine the capacity of the antioxidant compounds contained in the organic waste of the mealworm to capture the generated free radicals, thereby operating against the harmful effects of oxidation processes, involving reactive oxygen species (ROS).

It was found that the antioxidant capacity of this waste is comparable to those provided by conventional foods such as black tea, cranberry, tomato juice, among others.

This study provides scientific information to verify and endorse the potential benefits of using the waste of *T. molitor* larvae for the formulation of functional foods or to lead the extraction of the compounds it has and present it as a Nutraceutical.

Protective effect of achiote extracts against different types of stress in the model of *C. elegans*

Darío Gómez-Linton; Silvestre Alavez; Arturo Navarro-Ocaña; Luis Pinzón-López; Laura J. Pérez-Flores*.
Universidad Autónoma Metropolitana, Iztapalapa. Departamento de Ciencias de la Salud; CDMX, 09340, México. Tel. (55) 58046481; ljpf@xanum.uam.mx.

Introduction. Achiote (*Bixa orellana*) is a shrub native to tropical America,appreciated due it is a source of pigments of great commercial importance, the most abundant of them is bixin, an apocarotenoid present in the seeds that represents 90% of the pigments and up to 3% of the total weight of the seed. Besides, achiote seed is the main known source of tocotrienols. Both compounds have important antioxidant capacity. Antioxidant compounds could help living organisms to stay viable longer, preventing or delaying the occurrence of biomolecules damage that happens in oxidative stress.

Methodology. Achiote extracts were obtained by probe sonication using the green solvent isopropyl acetate. Extracts had a bixin and δ-tocotrienol content of 30% and 10% w/v respectively, being the main bioactive compounds. These extracts were tested on the N2 strain of C. elegans to know its effect on the lifespan curve and in resistance to thermal, oxidant and UV light stress. Additionally, strains DA465 (caloric restriction witness) and GA186 (deficient in SOD-3) was used.

Results and Discussion. Achiote extract showed protect nematodes against different types of stress, mainly at concentrations of 100 and 170 µg/ml. These two concentrations extended the half (28%) and maximum (12%) life of N2 strain. The protective effects against thermal and oxidative stress were conserved in DA465 and GA186 strains, which indicates that the effect is not given by caloric restriction (caused by nematodes do not feed on bacteria when the extract is added) and it is independent of SOD-3, one of the main antioxidant enzymes of the nematode.

Different types of stress have been related to the appearance of oxidative damage in biomolecules; bixin and δ-tocotrienol have antioxidant capacity, so they could act directly inhibiting or neutralizing radicals or indirectly by activation of survival signaling pathways to increase the nematodes lifespan and resistance to stress.

Conclusion. Achiote extract is able to increase half and maximum life of the *C. elegans* N2 strain, as well as to increase its resistance to different types of stress. This effect was maintained in the caloric restriction mimic strain and was independent of SOD-3. It is suggested that these effects are due to bixin and δ-tocotrienol, which are the major components with biological activity in achiote extracts.

Effect of hypoxia, reoxygenation, temperature and silencing on the expression of white shrimp *Litopenaeus vannamei* manganese superoxide dismutases

Carlos Ricardo González-Ruiz[a], Silvia Gómez-Jiménez[a], Alma Beatriz Peregrino-Uriarte[a], Elisa Miriam Valenzuela-Soto[a], Miguel Angel Martínez-Téllez[a], Francisco Javier Cinco-Moroyoqui[b] and Gloria Yepiz-Plascencia[a,*]
[a]Centro de Investigación en Alimentación y Desarrollo, A.C., Carretera a Ejido La Victoria Km. 0.6 Hermosillo, Sonora, 83304, México
[b]Departamento de Investigación y Posgrado en Alimentos, Universidad de Sonora, Blvd. Luis Encinas y Blvd. Rosales, Hermosillo, Sonora 83000, México
*Corresponding author: gyepiz@ciad.mx

The white shrimp *Litopenaeus vannamei* is one of the most important economic crustacean species. Its production comes from fisheries and aquaculture and in both conditions; it is exposed to environmental stress. Changes in temperature and oxygen availability can result in oxidative stress. This study investigated the effect of high temperature (35 *vs* 28 oC) and hypoxia (1.5 *vs* 6 mg O2/L) on the expression of two manganese superoxide dismutases, a cytosolic (cMnSOD) and a mitochondrial (mMnSOD) isoforms in *L. vannamei*. Also, the mMnSOD gene was silenced by RNAi to analyze the effect on the expression of both isoenzymes. In hepatopancreas, the expression of both MnSOD was induced at 35°C, 2.4 and 2.5 times for the mMnSOD and the cMnSOD respectively, respect to the control. In the same tissue, the expression was induced to a lesser extent during hypoxia 1.6 and 1.3 times for the mMnSOD and the cMnSOD and during reoxygenation, the expression was induced 2.4 and 7.0 times for the mMnSOD and cMnSOD. Also, an interaction effect of the heat and hypoxia stress factors together ($P < 0.05$) was observed on the expression of both MnSOD isozymes, where the expression was induced, 5.7 and 8.4 times for the mMnSOD and cMnSOD respectively, compared to the control. The expression was induced, 5.7 and 8.4 times for the mMnSOD and cMnSOD respectively, compared to the control. A decrease of 87.3 % in the *mMnSOD* transcripts in the control treatment was obtained in response to mMnSOD dsRNA injection, indicating efficient silencing. These results indicate that both MnSOD isoforms participate actively in the antioxidant response of the shrimp when exposed to high temperature and hypoxia.

ANALYSIS OF THE ACTIVITY OF PHENOLIC BEAN COMPOUNDS IN OXIDATIVE STRESS TOLERANCE IN *Caenorhabditis elegans*

*Alejandra Yitzel Guzmán Hernández, Marysol Rubio Landa, Josué Altamirano Hernández**
Laboratorio de Biología Sintética, Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Ciudad Universitaria, 58030, Morelia, Michoacán, México.
*Phone/Fax: (443) 2-99-01-81 ext. 511/ (443) 3-26-57-88 ext. 103 E-mail: josueah@hotmail.com

Key words: Oxidant stress, phenolic compounds, antioxidants.

Oxidant stress is a consequence of an imbalance between the production of free radicals and the antioxidant capacity of an organism. Antioxidants are molecules that deactivate free radicals and inhibits the initiation or propagation ofmfree radicals chain reactions. It has been shown that common bean (*Phaseolus vulgaris L.*) is a rich source of antioxidant substances, mainly phenolic compounds. These compounds have been shown to prevent chronic-degenerative diseases in which oxidative stress is generated. The objective of this work was to evaluate the biochemical changes in *Caenorhabditis elegans* that allow to elucidate the activity of phenols in the prevention of oxidative stress, which have been reported could prevent cancer and diabetes. The methanolic extraction of the seeds of commercial and landrace bean varieties was performed. The phenolic compounds were quantified by the Folin-Ciocalteu method. *In vitro* antioxidant activity was determined by the DPPH-, ABTS + and FRAP techniques. For the *in vivo* tests, the wild strain was used, as well as mutant and reporter strains of *C. elegans* used as an experimental model, which were subjected to oxidative stress by the addition of H_2O_2 and glucose in the medium. The results showed that the commercial bean had a total phenol concentration of 1.73 mg of EAG g-1, while the Creole had 3.47 mg of EAG g-1. *In vitro* antioxidant tests, for example, in the DPPH-test, the commercial pinto bean showed an activity of 375 µM of TROLOX mL-1 compared to 284 µM of TROLOX mL-1 of the Criollo. Interestingly, in vivo antioxidant assays demonstrated that Criollo bean extract had the best protective effect compared to the commercial one with a higher H_2O_2 LC50 and a 20% increase in survival. The effect of mutant strains of *C. elegans* in genes of interest to oxidant stress was analyzed, clearly observing that the synergy of bean phenolic compounds depends on the insulin signaling pathway which has been associated with this phenomenon and that The mixture of phenolic compounds found in beans has an indirect effect by modulating the oxidant stress response.

Resveratrol regulates oxidative markers and antioxidant enzymes during the aging process

Daniel Juárez Serrano (Estudiante de Licenciatura)[1,5]; Addí Rhode Navarro Cruz [1]; Eduardo Miguel Brambila Colombres [2, 4,5]; Alfonso Daniel Díaz Fonseca [3];Patricia Aguilar Alonso [1,4,5].
[1] Departamento Bioquímica-Alimentos, Facultad de Ciencias Químicas. BUAP; [2] Departamento de Análisis Clínicos, Facultad de Ciencias Químicas. BUAP; [3] Departamento de Farmacia, Facultad de Ciencias Químicas. BUAP; [4] Posgrado en Ciencias Químicas. BUAP; [5] Laboratorio de Investigaciones Químico-Clínicas. BUAP. Benemérita Universidad Autónoma de Puebla (BUAP)
14sur y San Claudio S/N, Col San Manuel CP 72570, 2295500 EXT 7483, 2861
agapmx@yahoo.com.mx

Aging is a natural process experienced by anyone living being, it includes morphological, functional and psychological changes and depends on various factors. Oxidative stress is an imbalance between oxidants and antioxidants leaning towards an oxidative environment, which potentially leads to molecular damage accelerating the appearance of the manifestations of aging. Because free radicals are physiological products of metabolism, they can be eliminated by antioxidant enzymes present in the cell such as: Superoxide Dismutase and Catalase, however, the activity of the endogenous antioxidant system is not always it is enough to counteract oxidative damage. The decline of the functioning of the cerebellum is related with the increase of oxidative stress during aging, producing dysfunctions in perception of time, precise movement, control of muscle tone and eye movements. Various antioxidant molecules have been described to counteract these effects, one of which is Resveratrol, a polyphenol with antioxidant, anti-aging, anticancer and neuroprotective properties. The aim of this work is to evaluate the effect of resveratrol on the lipoperoxidation products and the enzymatic activity of SOD and Catalase in the cerebellum of rat during aging process. Wistar Rats were divided into four groups: Control, Vehicle, Vitamin E and Resveratrol, submitted to a chronic administration during a period of 2, 4, 6 and 8 months, after that they were sacrificed and the cerebellum was obtained. Performed the analysis of nitrites, MDA + 4HDA, MDA, 4-HDA and enzymatic activity of Catalase and Superoxide Dismutase. The results showed that at the end of the different periods of administration with Resveratrol decrease nitrite levels in comparison with the control and vehicle groups, the MDA levels decrease significantly with the treatment with Resveratrol, the enzyme superoxide dismutase shown increased after 2 and 4 months of treatment with resveratrol compared to the control group and at 6 and 8 months was not modified, the activity of the catalase enzyme shows a tendency to decrease after 2 months of treatment, but after 8 months of treatment, its activity increases with the treatment. In conclusion, resveratrol presents an antioxidant effect in the cerebellum of rats treated by decreasing cellular lipoperoxidation and modifiyng enzymatic antioxidant activity

Stress-induced premature senescence (SIPS) of Primary Prostate Epithelial Cells (HPEC) and Evaluation of Senescence-Associated Secretory Phenotype (SASP)

Verónica Salas-Venegas[&*], Angélica Alejandra Aquino-Cruz[&*], Luis Ángel Maciel-Barón[&*], Rebeca López-Marure[#], Ma. Concepción Gutiérrez-Ruiz[&], Mina Königsberg-Fainstein[&], Norma Edith López-DíazGuerrero[&***]
[#]Departamento de Fisiología. Instituto Nacional de Cardiología "Ignacio Chávez".
*Posgrado en Biología Experimental, UAM Iztapalapa.
[&]Universidad Autónoma Metropolitana Iztapalapa, Av. San Rafael Atlixco 186, Leyes de Reforma 1ra Sección, 09340 Ciudad de México, CDMX. Tel. 5524937625 *** norm@xanum.uam.mx

Introduction. Aging is a biological phenomenon characterized by a progressive loss of the physiological integrity of the organism that leads to deterioration and vulnerability that finally leads to death. Significantly, in the tissues of old organisms and pathologies associated with aging, a progressive accumulation of senescent cells has been observed. Senescence is a cellular program of response to stress damage, where a stable arrest of cell proliferation accompanied by different phenotypic alterations is induced and with a particular secretome named "Secretory phenotype associated with senescence (SASP)". The SASP includes a variety of soluble and insoluble factors, among which are growth factors, interleukins, chemokines, and proteases, whose expression depends on the cell type and their physiological nature. In pathologies, the SASP contributes to cell proliferation, angiogenesis, and inflammation. In prostate pathologies, a large number of senescent epithelial cells have been observed in situ. To study the cellular and molecular mechanisms of senescence, cell culture models are useful tools. In particular, stress-induced premature senescence (SIPS) models have contributed to the identification of mechanisms involved in this process.
Methods. The cell culture of Primary Prostate Epithelial Cells; Normal, Human (HPEC) (ATCC®) was grown in Prostate Epithelial Cell Basal Medium supplemented with Prostate Epithelial Cell Growth Kit and 1% of an antibiotic-antifungal solution. The cell culture was incubated at 37 ° C and 5% of CO_2. The HPEC cultures were treated with different concentrations of H_2O_2 (12, 25 and 50 µM) for 2 hours to achieve the establishment of stress-induced premature senescence (SIPS). After the induction of senescence, on days 1,4, 8 and 12, cell proliferation was quantified by the trypan blue exclusion technique, as well as the percentage of senescent cells by SA-βgalactosidase activity. An immunocytochemistry probe was performed targeting p16, p21, p53, and β-gal to validate the senescent state. With a RayBio ® cytokine detection kit was analyzed the SASP of the cell cultures of days 4 and 9.
Results. The establishment of senescence in prostate epithelial cells was with the concentration of 12 µM of H_2O_2. The senescent state was validated by immunocytochemistry biomarkers of p16, p21, p53, and β-gal. Changes in SASP from senescent HPEC were observed compared to non-senescent HPEC with an increment of IL-6 and IL-8.
Conclusions. We were able to establish a SIPS model in vitro of Primary Prostate Epithelial Cells of human prostate with a concentration of 12 µM of H_2O_2. The expression of SASP of senescent cells compared to non-senescent cells indicates that the SASP could participate directly in various cellular processes such cell proliferation of neighboring cells.

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Low-intensity and long-term training prevents sarcopenic obesity in female Wistar rats

Beatriz Mena-Montes [1,2,7], Norma Edith López-DiazGuerrero [2] , María del Rocio González-Vieira [3] , Roberto Lazzarini-Lechuga [4] , Oscar Rosas-Carrasco [5] , Gibrán Pedraza-Vázquez [1,2], David Hernández-Álvarez [2], Alfredo Morales-Salazar [2] , Ulalumen Hernández-Arciga [2], Alejandro Silva-Palacios[1,2], Sandra Morales-Rosales[1,2], Rafael Toledo Pérez[1,2], Edith Hernández Cruz [2], Luis Enrique Gómez-Quiroz [6] , Mina Konigsberg Fainstein[2] , Armando Luna-López [7*]

[1]Posgrado en Biología Experimental, DCBS, Universidad Autónoma Metropolitana Iztapalapa. [2]Laboratorio de Bioenergética y envejecimiento celular, Departamento de Ciencias de la Salud, Universidad Autónoma Metropolitana Iztapalapa. [3]Bioterio, Universidad Autónoma Metropolitana Iztapalapa. [4]Departamento de Biología de la Reproducción, Universidad Autónoma Metropolitana Iztapalapa. [5]Depto. Investigación Clínica-Epidemiológica, Instituto Nacional de Geriatría. [6]Laboratorio de Fisiología Celular, Departamento de Ciencias de la Salud, Universidad Autónoma Metropolitana Iztapalapa. [7]Departamento Investigación Básica, Instituto Nacional de Geriatría.
*Corresponding author: Armando Luna López. allbioexp@yahoo.com
Dirección Avenida Contreras 428, colonia San Jerónimo Lídice, Delegación Magdalena Contreras, Ciudad de México, C.P. 10200. Tel. 56551921

Sarcopenic obesity (SO) is a medical condition defined as the presence of both sarcopenia and obesity. Sarcopenia is the gradual loss of strength, quality, and quantity of muscle during aging, and obesity is associated with an excess of body fat. According to the European Working Group on Sarcopenia in Older People (EWGSOP), the Dual-energy X-ray absorptiometry (DXA-scan) is the gold standard for diagnosis for SO.

Our interest was to evaluate the level of sarcopenic obesity of Wistar of sedentary rats of 4, 8, 12, 18, 22 and 24 months old, compared to rats of the same ages under a low-intensity exercise regimen.

Groups were compared using the DXA-scan, histological analysis, and oxidative protein damage.

Our preliminary results showed significant differences ($p < 0.05$) in the composition of fat mass (FM), fat mass index (FMI), fat-free mass index (FFMI), FM/FFM, fat-free and bone-free mass (FFBFM) and protein oxidative damage between groups. Hematoxylin and eosin staining revealed morphological changes in the proportion of fat in comparison to a sedentary lifestyle.

Our results provide a good model for the molecular study of SO and oxidative stress in aging that could be a used for the evaluation and comparison of therapeutic strategies for effective SO prevention.

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The cardioprotective effect of sulforaphane is mediated trough an Nrf-2 independent pathway.

Marcos Ostolga Chavarría., Alejandro Silva Palacios., Ana Cecilia Zazueta Mendizábal.
Instituto Nacional de Cardiología I.Ch. Departamento de Biomedicina Cardiovascular.
Calle Industria Militar L.9 Mnz. L Col. San Fernando Estado de México, Tel. 5576567865 correo: m_och@outlook.com

The only treatment against acute myocardial infarction (AMI) is early reperfusion of the occluded coronary artery; however, this procedure may induce additional injury to that producer during ischemia, contributing to increase the final size of the infarcted myocardial area. Different studies have provided information on the role of reactive oxygen species (ROS) in the pathogenesis of reperfusion injury. In consequence, several investigations have focused on the attenuation of oxidative stress produced during reperfusion. (1) In this work we evaluate the effect cardioprotective of Sulforaphane (SFN, 1-isothiocyanato-4 – [(R) -methylsulfinyl] – butane), which plays a key regulator role in cellular defense, through the induction of the transcription factor Nrf2 (Nuclear factor-erythroid-2- (NF-E2-) related factor 2) this factor controls the expression of a wide variety of genes that code for proteins of the endogenous antioxidant system and phase II enzymes. (2) In addition, SFN has also been pointed out as and inducer of autophagy and inhibitor of inflammation, mechanisms which might contribute to diminish reperfusion damage.

Objective

In this project we studied the mechanisms by which SFN exerts cardioprotective effect in ischemic-reperfused hearts and compared its efficiency against the maneouver of postconditioning, which activates cellular signaling mechanisms that lead to heart protection.

Results

The administration of both SFN [500 µg / kg] and the application of PostC preserved the function and architecture of cardiac tissue, in association with reduced oxidative damage to macromolecules in ischemic-reperfused hearts. We also observed that although both treatments activate Nrf2 upstream kinase signaling, SFN activates alternative effector to Nrf2 as compared to PostC.

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Differential effects of carbohydrates and fatty acids on oxidative stress and mitochondrial alteration in a hypothalamic cellular line

Itzel Pérez Ayala, Minerva Ramos Gómez, Alberto Camacho Morales, Rosalía Reynoso Camacho, Eduardo Castaño Tostado, Luis Alberto Madrigal Pérez.
Departamento de Investigación y Posgrado en Alimentos, Facultad de Química, Universidad Autónoma de Querétaro. Profesor-Investigador encargado Dra. Minerva Ramos Gómez. Tel: (442) 192-1200, 192-1307 Ext 557. Correo electrónico: ramosgomezm@yahoo.com. C.U., Cerro de las Campanas Querétaro, Qro. 76010

Energy homeostasis involves the control of food intake and caloric expenditure, bidirectional regulated by the central nervous system and peripheral organs interactions, where the hypothalamus is a key brain structure on this interaction. However, under positive energy balance such as during uncontrolled consumption of carbohydrates and lipids, the hypothalamus might be a target of toxicity leading to neuronal oxidative stress activation and mitochondria dysfunction. However, the role of selective nutrient overload on mitochondria-dependent energy homeostasis during obesity is unknown. In this project the effect of increased carbohydrates and fatty acids, such as glucose (GLU), fructose (FRU), palmitic (PA) and oleic (OA) acids, on oxidative stress and mitochondrial function was studied using the mHypoA-CLU192 hypothalamic cell line. Oxidative state was evaluated by analysis of antioxidant system activation markers such as glutathione (GSH), where a 50-80 % increase was achieved at the two lowest concentrations of FRU and the highest of GLU and both fatty acids. Similarly, the activity of glutathione peroxidase (GPX) increased at the highest level of GLU, FRU, PA and OA, while the release of hydrogen peroxide (H2O2) did not show significant variations. In addition, we observed a cytotoxic effect at high FRU and PA concentrations, and no changes in cellular viability at any GLU concentration, whereas OA exerts a dose-independent cytoprotective effect. Finally, mitochondrial function was evaluated through the ADP/ATP ratio and oxygen consumption rate. We found a significant 60-70 % and 30% ATP decrease after carbohydrates and fatty acids stimulation, respectively, when compare with control. Based on these results, GLU, FRU, PA and OA overload play a significant role on oxidative stress activation in brain cells; whereas a differential nutrient-depending effect on mitochondrial function was observed. We propose that selective oxidative stress and mitochondrial function alterations by nutrients might be potential modulators of metabolic homeostasis settings such as food intake or energy expenditure.

Key words: Hypothalamus, carbohydrates, fatty acids, oxidative stress, mitochondria

Effect of 17-β estradiol and progesterone on the evolution of Myocardial Infarction

Authors: M.Sc. Diana Ramírez Hernández[1-2], Ph.D. Jazmin Flores Monroy[1], Ph.D. Pedro López Sánchez[2], Ph.D. Luisa Martínez Aguilar[1], Ph.D. Martha Cecilia Rosales Hernández[3]. Universidad Nacional Autónoma de México- Facultad de Estudios Superiores Cuautitlán, Laboratorio de Farmacología del Miocardio[1]. Instituto Politécnico Nacional- Escuela Superior de Medicina, Laboratorio de Farmacología Molecular[2], Laboratorio de Biofísica y Biocatálisis[3].

Av. 1 de Mayo S/N, Colonia Santa María las Torres, Cuautitlán Izcalli, Estado de México, México. CP. 54740. Tel: 5556232049. jfmqfb@yahoo.com.mx

The incidence of myocardial infarction (MI) in pre-menopausal women is low compared with men of the same age range, however, in post-menopausal women it reaches the same frequency or even exceeds that of men. Various studies have shown that sex hormones play a cardioprotective role in women. The aim of this project was to determine the differences in the evolution of myocardial infarction due to the variation of sex hormone levels in the model of coronary occlusion in female Wistar rat, through the quantification of NO and reactive oxygen species. **Methods:** 63 female Wistar rats were used and grouped as: 1) Sham-estrus, 2) Sham-Proestrus, 3) Sham-Ovx; Coronary occlusion (CO) groups: 4) 48 h-estrus; 5) 2 weeks-estrus; 6) 48 h-proestrus; 7) 2 weeks-proestrus; 8) 48 h-ovx; 9) 2 weeks-ovx. To all groups the levels of the NO and ROS of the left ventricle by Griess test and EPR respectively were obtained. Also infarct area, cardiac hypertrophy, systolic and diastolic blood pressure, cardiac frequency, systolic and diastolic left ventricle pressure and the plasma levels of the estradiol and progesterone, were evaluated. **Results:** Female Wistar rats in proestrus phase at 48 h and 2 weeks of CO showed significant decrease of infarct area in comparison with estrus and ovx groups, no changes were observed in hypertrophy index and cardiac frequency. A decrease in systolic and diastolic blood pressure in female rats in estrus, proestrus phase and ovx surgery at 48 h of CO was found. The diastolic left ventricle pressure showed a significant increase in rats in estrus and proestrus phase and ovx surgery at 48 h of CO. Sham-ovx and 48 h-ovx groups showed significant increase of NO levels in comparison with estrus and proestrus groups; 2 weeks-estrus group showed a significant increase of NO in comparison vs other groups at same CO time. ROS levels decreased in proestrus gropus on all CO evolution times in comparison with ovx and estrus groups. **Conclusion:** Our findings indicate that exist a higher cardioprotective effect in female Wistar rat during proestrus phase wich is mediated by elevation of estrogens. **Acknowledgement:** This study was supported by PROYECT PAPIIT IN213318 DGAPA-UNAM; PIAPI 1828 FESC-UNAM; PIAPIME 2018 10.2.11.02.18 FESC-UNAM

Moringa oleifera extract attenuates alloxan-induced metabolic changes through suppressing the iNOS expression and modulating eNOS activity in heart and liver of rats.

[1]Cristina Ramos Olivas, [1]Mónica Andrea Valdez Solana, [1]Gladys Cithlaly Escareño Alcalá, [2]Guadalupe García Arenas, [1]Erick Sierra Campos*

[1]Facultad de Ciencias Químicas de la Universidad Juárez del Estado de Durango, Campus Gómez Palacio. Durango. Av. Artículo 123 S/N Fracc. Filadelfia. Gómez Palacio, Dgo. CP. 35010. *Email: ericksier@gmail.com
[2]Facultad de Ciencias de la Salud de la Universidad Juárez del Estado de Durango, Campus Gómez Palacio. Durango.

Abstract

Vascular effects of flavonoids have been associated with nitric oxide availability. flavonoids have been reported to modulate pro-oxidative enzymes, resulting in decreased reactive oxygen species (ROS) production and consequential increased nitric oxide (NO). In addition, several *in vitro* and human studies have reported potent vasorelaxant activity of certain flavonoids related to activation of endothelial Nitric Oxide Synthase. This supports the importance of flavonoids in eNOS activation and NO availability. There is accumulating evidence to support benefits of *Moringa oleifera* phytochemicals on cardiovascular and hepatic health. Furthermore, hepatoprotective effect of *M. oleifera* have been consistently reported in animals. However, it is difficult to identify the specific mediators of health due to the numerous potential bioactive compounds present in *M. oleifera* leaves. We previously described a hepaprotective role of a treatment with *M. oleifera* leaves extract in diabetic rats. The aim of this study was investigated the effects of this treatment on different isoforms of nitric oxide synthase (inducible NOS and eNOS) and oxidative stress in diabetic heart and liver. Fourteen Wistar rats were randomly selected into three groups; three rats received saline solution in the control group, five rats in diabetic group received a single dose (120 mg/Kg b.w, i.p.) of alloxan, and group DM containing six rats received *M. oleifera* extract treatment (200 mg/kg b.w, p.o.). Nitrate, glucose, triglycerides and cholesterol levels, catalase, nitric oxide synthase and paraoxonase^{-1} (PON1) activities were analyzed after four weeks. The *M. oleifera* extract produced significant reduction ($P < 0.05$) in blood glucose and had triglycerides. Nitrates levels was reversed near normal in treated rats as compared to diabetic control. However, there was no significant difference in PON1 activity between treated rats as compared to diabetic control. We concluded that an *M. oleifera* extract is effective in controlling blood glucose levels and improves eNOS activity mainly in heart.

Keywords: Alloxan, *Moringa oleifera*, eNOS, PON1, diabetes

Synthesis and characterization of antioxidant compounds and inhibitors of myeloperoxidase derived from cinnamic acid.

Astrid Mayleth Rivera Antonio[1-2],Martha Cecilia Rosales Hernández[2], Itzia I. Padilla Martínez[1]
[1]Laboratory of Supramolecular Chemistry and Nanosciences, Department of Basic Sciences, Interdisciplinary Professional Unit of Biotechnology, National Polytechnic Institute, Av. Acueducto s / n, Barrio La Laguna Ticomán, Mexico City 07340, Mexico, [2]Laboratory of Biophysics and Biocatalysis, Studies Section of Postgraduate and Research, Higher School of Medicine, National Polytechnic Institute, San Luis Plan and Salvador Diaz Mirón s / n, Casco de Santo Tomás, Mexico City 11340, Mexico Email: ASTRIDMRIVERAA@gmail.com Telephone: 5548123266

Introduction: Oxidative stress is associated with chronic-degenerative diseases such as cancer, Parkinson's, Alzheimer's and atherosclerosis1-2, due to the damage caused to macromolecules such as proteins, lipids and nucleic acids. During these diseases Myeloperoxidase (MPO) produce high amount of hypochlorous acid (HOCL) contributing to the macromolecules damage. Antioxidants have been studied for the benefits they provide in the prevention of chronic-degenerative diseases. The molecules with antioxidant activity are phenolic acids, flavonoids, tannins and coumarins.1 Objective: to synthesize compounds derived from cinnamic acid with possible antioxidant activity and myeloperoxidase inhibitor. Methods: an organic synthesis was carried out assisted by microwaves, to obtain 10 compounds derived from cinnamic acid, which were evaluated with the DPPH and ABTS antioxidant assays, and as MPO inhibitors (peroxidation and chlorination). Results: The synthesized compounds presented antioxidant activity of up to 90% and inhibitor of 70%. Discussion: because the radical ABTS is less stable than DPPH, it reacts faster, so a decrease in the radical was observed with a lower concentration of compound, compared with DPPH. For the enzymatic inhibition, the synthesized compounds had better activity in peroxidation. The antioxidant activity of the compounds is related to their functional groups, which give them the ability to donate hydrogen atoms to unstable molecules, such as reactive species. Because of its structure, this group of molecules contains in its structure hydroxyl groups and aromatic rings which favor their activity as antioxidants and inhibitors of myeloperoxidase. Conclusion: The strategies used for the synthesis of compounds were adequate since a good yield of the compounds was obtained. The compounds presented antioxidant activity similar to resveratrol which was used as a reference; however, its activity was not similar to that of 5-ASA and TROLOX.

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Fatty acids promoted oxidative stress in *Yarrowia lipolytica* cells

Sandra Lucía Rodríguez-Teniente, Gerardo I. Arredondo-Mendoza, Blanca Edelia González-Martínez, Mariela Escalera-Elizondo, Maripaz Castillo-Roque, Celinda Kim- Mandujano, Zacarías Jiménez-Salas, Eduardo Campos Góngora.

Laboratorio de Proteómica, Facultad de Salud Pública y Nutrición, Universidad Autónoma de Nuevo León.
Address: Dr. Eduardo Aguirre Pequeño S/N, Mitras Centro, 64460 Monterrey, N.L.
Phone: (81) 1340 4890 Ext: 3062. Email: sandrardzteniente@hotmail.com

Introduction. The majority of living organisms depend on oxygen for survival; however, reactive oxygen species (ROS) may affect the cell metabolism. Oxidative stress (OS) is the imbalance between the amount of ROS and the ability of antioxidant protection systems to counteract their action. The excessive generation of ROS can cause cellular damage contributing to the development of a large number of pathologies. There is a significant relationship between the intake of fatty acids and the development of OS.

Objective. To analyze the effect of oils with a high content of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) on the generation of oxidative stress in *Yarrowia lipolytica* cells.

Materials and methods. *Y. lipolytica* cells (strain P01A) were cultured in different media (YP and minimum medium YNB) added with 2% of: SFA (coconut oil), MUFA (canola oil) and PUFA (chia oil). Cell growth was monitored during different times using a spectrophotometer (OD600). The amount of ROS produced by cells growing in the different media, was quantified by fluorescence using a fluorimeter (Fluoroskan Ascent). The results were compared by one-way ANOVA.

Results. In both culture media (YP and YNB) there was an increase in the growth of the cells cultured with MUFA, while with SFA a lower growth was observed ($p=0.009$). The cultures with PUFA did not show differences in their growth compared to the control (added with glucose, as carbon source). Significant differences were found in the generation of ROS in the culture media at certain times. The greatest amount of ROS was found in the culture media with PUFA, and the lowest in the media with MUFA.

Conclusion. In *Y. lipolytica* cells, ROS generation and growth rate depend on the fatty acids used as carbon source.

Association of telomere length, oxidative stress and frailty in an elder population

José Darío Martínez-Ezquerro[1], Aleida Rodríguez-Castañeda, Mauricio Ortiz-Ramírez, Sergio Sánchez-García, Haydee Rosas-Vargas, Rosalinda Sánchez-Arenas, Paola García-delaTorre*

Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social (IMSS)
[1]Unidad de Investigación Médica en Genética Humana
*Unidad de Investigación Médica en Enfermedades Neurológicas, Av. Cuauhtémoc 330, Col. Doctores, CDMX, México; +5215541455279; pgarciatorre@gmail.com

Abstract

Background & objectives: Public health challenges from global population aging requires to focus research on the risk factors for unhealthy aging, preventive medicine and chronic disease management. Frailty is a leading cause of functional decline and mortality in older people. This condition has been characterized as a biological syndrome associated with diminished strength, reduced gait speed, and exhaustion. On the other hand, oxidative stress and telomere shortening have been suggested as biological biomarkers of aging. Here we evaluated the association of oxidative stress, telomere length and frailty in an elderly population of Mexico City.

Aim of the study: To determine the association between oxidative stress (measured by reactive oxygen species (ROS) and lipid peroxidation), telomere length, and frailty in a Mexican elder population.

Methods: This cross-sectional study was based on 2015 data from the Cohort of Obesity, Sarcopenia and Frailty of Older Mexican Adults. Frailty was diagnosed using criteria proposed by Fried. We measured ROS by dichlorofluorescein diacetate and lipid peroxidation by malondialdehyde, while telomere length was determined by qPCR.

Results: Contrary to our expectations, we found no effect of oxidative stress on telomere length or frailty; however, we found a significant association between telomere length and frailty.

Interpretation & conclusion: Oxidative stress, measured only as ROS and lipid peroxidation, seems to reach a homeostatic level in our elderly population which has no effect on telomere length or frailty status. On the other hand, shorter telomeres were associated with frailty, an accurate identifier of health outcome. Hence, telomere length measurements need further research as a possible biomarker to sieve between healthy and unhealthy aging, including frailty diagnosis.

Key words: aging; frailty; oxidative stress; telomere length

Does the antioxidant system and ABA are involved in aluminum tolerance during the first hours of treatment in *Fagopyrum esculentum* seedlings?

Ana Violeta Salazar-Chavarría and Rocío Cruz-Ortega
Instituto de Ecología, UNAM, Circuito Exterior Universitario S/N anexo Jardín Botánico Exterior Ciudad Universitaria, Ciudad de México, C.P. 04500
rcruz@ecologia.unam.mx

Aluminum toxicity (Al) is one of the major constraints for plant growth on acid soils. While most plants are sensitive to Al, some species have developed strategies to cope with Al toxicity. *Fagopyrum esculentum*, var Mancan (Polygonaceae) regardless is an aluminum tolerant plant, as a seedling, showed root inhibition during the first hours of exposure to Al, and then it fully recovered. In this study we assessed if the antioxidant system and ABA might be involved in *F. esculentum* tolerance mechanisms. Thus, we evaluated in roots of seedlings of 3 days old exposed to 50 µM Al for 3, 6, 12, 24 and 48-h; relative root growth (RRG); entrance of Al to the root by a modified hematoxylin stain; root cell viability with fluorescein diacetate and propidium iodide, endogenous ABA level by immunoassay method; levels of ROS by fluorescence (DCFDA); the activity of cytosolic catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and ascorbate peroxidase (APX) by spectrophotometric method; and the effect of exogenous ABA to evaluate the activity of CAT, APX, GR and ROS production.
Results showed that RRG is inhibited during the first 12-h of Al-treatment, but at 24-h and 48-h the RRG started to recover compared to control seedlings (50%). Al enters and accumulated into roots since 3-h up to 24-h, and then the level of Al remained unchanged. CAT, GR and APX activity increased from 6 to 48-h of Al-treatment, as well as ROS, however there was not cell damage at root tip. Endogenous ABA levels increased significantly in Al root treated. Finally, the application of exogenous ABA increased the CAT and GR activity and elevates ROS production. All together, these results suggest that during the first 12-h of Al treatment, there is a decline in root growth and an increase in ROS levels due to Al entrance to the root. However, the increase in antioxidant activity allows the recovery of root growth, and this effect might be mediated by ABA.

Participation of Iba57p in the [2Fe-2S] cluster assembly of the Rip1 subunit into the cytochrome *bc1* complex from *Saccharomyces cerevisiae*

Luis Alberto Sánchez Briones, Mauricio Gómez Gallardo, Alma Laura Díaz Pérez and Jesús Campos García
Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Av. Universidad s/n, C.P. 58030, Edif. B-3, Ciudad Universitaria, Morelia, Michoacán, México.
Phone: (443) 3223500, ext. 4214. jcgarcia@umich.mx; biol.luis.22@gmail.com

Abstract: The iron-sulfur clusters (Fe-S) are inorganic cofactors found in enzymes involved in different cellular processes. The simplest Fe-S clusters are the rhombic [2Fe-2S] and the cubic [4Fe-4S] types, their biosynthesis is a conserved process among kingdoms. In eukaryotes, Fe-S synthesis mainly occurs in the mitochondrial matrix by the ISC (Iron Sulfur Cluster) assembly system. This system involves two steps: the de *novo* assembly of a Fe–S cluster on scaffold proteins, and the transfer of the Fe–S cluster from the scaffold system to the target apo-proteins and its subsequent assembly into the polypeptide. These steps involve the participation of several proteins, which perform specific reactions, however their function have not been described completely. The ISC subsystem (Fe-S-IBG) conformed by the Grx5p, Isa1p, Isa2p, and Iba57p proteins has been proposed. This subsystem had been related with the synthesis of the [4Fe-4S] clusters. Yeast mutants in the Fe-S-IBG subsystem showed deficiency in respiration and excessive ROS generation, similarly with the mutant in the *RIP1* gene, which codes for the Rieske subunit of cytochrome *bc1* complex. In this work, the activity of cytochrome *bc1* complex in mitochondria from the *iba57Δ* and *rip1Δ* mutant strains were determined. Results showed that the cytochrome *bc1* complex on both mutants was dysfunctional. Immunodetection assays showed taht the Rip1 protein was absent in the mutant *iba57Δ* indicating that Iba57p is involved in the insertion of the [2Fe-2S] cluster on the Rip1p, suggesting that the Fe-S-IBG subsystem is not exclusive for the maturation of proteins with [4Fe-4S] clusters but also in [2Fe-2S] clusters such as in the Rieske subunit.

Keywords: iron-sulfur cluster; Fe-S-IBG subsystem; cytochrome *bc1* complex.

Sulforaphane effects on nerve conduction velocity and brain cortex and hippocampus redox state of old male and female Wistar rats

Roberto Santín Márquez[1,5], Alejandra Ibañez Contreras[3], Rafael Toledo Pérez[1,5], Belén Ramírez Cordero[1,5], Juan Ortíz Retana[4], Martín García Servín[4], Beatriz Gómez González[2], Mina Königsberg Fainstein[1]

[1] Laboratorio de Bioenergética y Envejecimiento Celular, Dpto de Ciencias de la Salud, Universidad Autónoma Metropolitana Unidad Iztapalapa. [2] Área de Neurociencias, Dpto de Biología de la Reproducción, Universidad Autónoma Metropolitana Unidad Iztapalapa. [3]APREXBIO S.A. De C.V. [4]Instituto de Neurobiología, Campus Juriquilla, UNAM, [5] Posgrado En Biología Experimental, Universidad Autónoma Metropolitana Unidad Iztapalapa

e-mail: robertosanmar23@gmail.com; phone number: 01 55 65275098

Aging is a multifactorial, universal, progressive and deleterious process, which is time-depending in the living beings. Its main feature is a physiological function decline and increased vulnerability that finally, trigger the organism death. Brain is one of the more sensible organs that are damaged during aging, because of its susceptibility to the aging-related oxidative stress. Thus in this study, the relation between the nerve conduction velocity, given by myelination and sensory nerve pathways integrity, was evaluated. Furthermore, this relation was linked with the brain cortex and hippocampus redox state, antioxidant enzymes function, and protein oxidative damage in young (4 months) and old (21 and 24 months) male and female rats. At 21 months of age, animals were treated with sulforaphane (SFN) for 3 months. Evoked potentials were performed for the auditory, visual, median and tibial somatosensory pathways. In both, males and females, the latencies were larger in the old rats groups in almost all waves; nevertheless, the SFN appears to have a protector effect on the nerve conduction velocity of all sensory pathways only in males, however the number of animals that achieved the 24 months of age was very reduced. In the morphometric magnetic resonance images assays (MRI) a decrease in the total volume of the brain cortex was detected in the old rats groups, but no difference was found in the hippocampus volume. There was not apparent effect of SFN in the total volume of neither brain cortex nor hippocampus.

Regarding to the redox state, the superoxide dismutase (SOD) and catalase (CAT) activity were not different between the young rats and the old rats groups, even with the SFN treatment. This could suggest that the protection effect given by SFN on the nerve conduction velocity might be related to an alternative pathway, different of the classical Nrf2 pathway, which improves the brain redox state.

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Effect of exposure to low doses of ozone on the expression of IL-17A during the process of progressive neurodegeneration in the hippocampus of rats

Helena Solleiro-Villavicencio*, Eduardo Hernández-Orozco* and Selva Rivas-Arancibia*
*Departamento de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México. Avenida Universidad 3000 ZIP code 04510, tel. 5591859593, e-mail: helenasolleiro@gmail.com, srivas@unam.mx

Abstract

Ozone is one of the main pollutants in the air. It has been described that the inhalation of this gas produces a state of oxidative stress, which is considered a critical factor in the development of neurodegenerative diseases. Previous studies in our work group showed that chronic exposure to low doses of ozone induces a state of oxidative stress and irreversible progressive neurodegeneration, that are accompanied by the loss of the regulation of the inflammatory response, in which cytokines play an important role. Recently, we reported that ozone produces an increase of IL-17A in the hippocampal tissue during the irreversible neurodegenerative process. However, we still do not know the cellular source from which IL-17A is secreted.

Thereby, the aim of this study was to evaluate the effect of chronic exposure to low doses of ozone on the concentration of IL-17A and its expression in neurons, microglia, astrocytes and T cells in rat hippocampus. For that purpose, we used 72 Wistar rats, divided into 6 groups (n = 12): control group (without ozone) and groups exposed to ozone (0.25 ppm, 4 h daily) for 7, 15, 30, 60 and 90 days, respectively. Six subjects from each group were processed to quantify IL-17A by ELISA, and the remaining 6 were processed for immunohistochemistry (against IL-17A and GFAP, Iba-1, NeuN or CD3).

The data obtained by ELISA test showed a significant increase in the concentrations of IL-17A in the groups of 7, 15, 30 and 60 days of exposure, compared with the control (P <0.05). Furthermore, they indicate that hippocampal neurons are the cells that showed the greatest immunoreactivity against IL-17A between 60 and 90 days of exposure to ozone, as well as an increase in activated astrocytes in groups of 30 and 60 days of exposure.

We conclude that exposure to low doses of ozone induces an increase in the expression of IL-17A, mainly in hippocampal neurons, accompanied by the activation of astrocytes in hippocampus of rats, as part of the loss of the regulation of the inflammatory response that takes place during the process of chronic neurodegeneration, similar to what occurs in Alzheimer's Disease in humans.

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Malondialdehyde and carbonyl levels in Wistar rats treated with streptozotocin and sugar water in neonatal age

Sergio Trinidad Rodríguez, Idaily Nava Garay, Martha Isela Barragán Bonilla, Juan Miguel Mendoza Bello, Mónica Espinoza Rojo.

Laboratorio de Genómica y Biología Molecular, Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Guerrero. Av. Lázaro Cárdenas s/n, Ciudad Universitaria Sur, Chilpancingo de los Bravo, Guerrero. Teléfono 7472207110. E-mail: sergio3pk@gmail.com

Introduction: Hyperglycemia and insulin resistance are main characteristics of diabetes mellitus 2 (DM2). In a hyperglycemic state during glucose metabolism high amounts of reactive oxygen species (ROS) are produced. ROS induces damage to lipids and proteins (oxidative damage), affecting various organs and generating diabetic complications. To determinate the relationship between hyperglycemia and oxidative damage, biomarkers have been determined to evaluate levels of lipid peroxidation and protein carbonylation, such as malondialdehyde (MDA) and carbonyl groups respectively. Several experimental models have been used to study DM2, among them; the administration of streptozotocin (STZ) in neonatal age, due this drug produces destruction partial of the cells of pancreas, generating hyperglycemia and insufficient progressive secretion of insuline. In other studies, supplementation of sugar water, it has shown to have hyperglycemic effect and contributes to the development of oxidative stress.

Objective: Evaluate the levels of MDA and carbonyl groups in plasma of rats treated with STZ in neonatal age and supplemented with sugar water during their development.

Material and methods: In this study were used male and female Wistar rats. Six study groups were formed. 1) Group without treatment (control); 2) group with 30% sugar water for 7 weeks (Ctrl 30% 7S); and 3) 11 weeks (Ctrl 30% 11S); 4) group treated with STZ (70 mg/kg of STZ at 2 days of age) (STZ); 5) group treated with STZ and 30% sugar water for 7 weeks (STZ + 30% 7S); and 6) 11 weeks (STZ + 30% 11S). At 14 weeks of age, plasma samples were obtained and the level of MDA and carbonyl groups was determined by spectrophotometry. To measure the plasma MDA was used with 1-methyl-2- phenylindole and the carbonyl groups were determined with Dinitrophenylhydrazine. The results were normalized with the concentration of plasma proteins total in plasm.

Results: In males, treated with STZ and supplemented with sugar water, a tendency to progressive increase in MDA levels was observed with respect to control. The levels of carbonyl groups, in males and females, no showed changes

Conclusion: STZ and chronic exposure to high levels of sugar in the diet, contribute to oxidative damage to lipids.

Evaluation of the enzymatic activity of catalase in a model of cancer treated with extracts of *Crotalaria retusa*

José Roberto Velázquez Murillo [1], Ernesto Mendoza-Vallejo [1], Catalina Machuca Rodríguez [1.]

FES Zaragoza, UNAM. Batalla 5 de mayo s/n esquina Fuerte de Loreto, Col. Ejército de Oriente, Iztapalapa C.P. 09230, Ciudad de México. catalina.machuca@gmail.com

Catalases perform the dismutation of hydrogen peroxide in water and dioxygen, preventing the formation of the hydroxyl radical and mono oxygen, oxygen species that are very reactive. In humans, catalase protects hemoglobin from hydrogen peroxide generated by erythrocytes, they have a protective role in inflammation, in the prevention of mutations, prevents aging and certain types of cancer. *Crotalaria retusa* is an herbaceous, tropical plant with a high content of secondary metabolites. Within the genus the presence of pyrrolizidine alkaloids has been described, in which its antibiotic activity and toxic effects have been studied. However, its cytotoxic activity in breast cancer has not been reported. In this study, the effect of aqueous and hydroalcoholic extracts of *C. retusa* on the activity of catalase in liver and mouse blood was evaluated. 3mL of the substrate and 25µL of sample were placed, immediate readings in a UV spectrophotometer at 240 nm, the absorbance obtained every 10 seconds was recorded during 1 minute. In the liver, the aqueous extract of the whole plant shows a reduction in the activity of catalase with respect to the control induced with DMBA (7, 12-Dimethylbenz[a]anthracene), on the contrary, the aqueous leaf extract indicates an increase in the activity of the enzyme, being superior to the control with DMBA. The plasma activity of catalase is reduced in response to aqueous leaf and whole plant extracts. The aqueous stem extract maintains high levels of catalase. The greatest decrease in catalase activity was observed in liver with the whole plant,and in plasma with aqueous stem.

Autophagy could cooperate with matrix metalloproteinases to the mouse neural tube closure

Pilar Sarah Acevo-Rodríguez, Sandra Cabrera-Benítez, Diana Escalante-Alcalde y Susana Castro-Obregón*
Departamento de Neurodesarrollo y Fisiología, División de Neurociencias, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México (UNAM). Circuito Exterior S/N Ciudad Universitaria, Coyoacán, 04510 Ciudad de México, D.F. Tel (52) (55) 5622 5676.
*scastro@ifc.unam.mx

In vertebrate development a crucial event occurs just after embryo gastrulation, the central nervous system primordium arises from a structure known as the neural tube. During neurulation process, at the dorsal portion of the embryo, the neuroectoderm thickens and flexes at a medial hinge point in response to signals released from the notochord, forming two concave walls that extend along the embryo"s anterior-posterior axis. Next, a second flexion occurs in the dorso-lateral portion of the walls causing their tips to fuse at the midline and forming a hollow cylinder known as neural tube. If the neural tube does not form properly, born defects occur like anencephaly and spina bifida that have a high prevalence in newborns.

Both, autophagy and matrix metalloproteinases have been associated with events related to neurodevelopment like neurogenesis, neuronal migration and myelination, but their role during early neural development is still unclear. Although the absence of activating autophagy protein (AMBRA1) causes lethality and neural tube defects in mouse embryos, no study has proven whether autophagy, and no other unknown function of AMBRA1, indeed contributes to the fusion of the neural tube nor which could be the mechanism.

Autophagy has been described as a lysosomal catabolic process that can also mediate non-conventional secretion of proteins. We hypothesize that during the neural tube closure autophagy mediates the unconventional secretion of matrix metalloproteinases, considering that these proteases are key elements for cellular events implicated in tissue fusion as celullar migration, proliferation, fusion and death. We observed cells with increased autophagic activity and expression of several matrix metalloproteinases at the neural folds of the neural tube in mice embryos. We will discussed the effect of inhibiting autophagy during ex-utero development.

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Association of miR-21 and let-7 with migration and invasion of medulloblastoma cells

Lourdes Álvarez-Arellano[1]*, Fabiola Ruiz-Madrigal[1]; Sandra Bordenave-Encarnación[1]; Pilar Eguía-Aguilar[2]; Carmen Maldonado-Bernal[3]
[1]Laboratorio de Investigación en Neurociencias, [2]Departamento de Patología, [3]Laboratorio de Investigación en Inmunología y Proteomica, Hospital Infantil de México Federico Gómez. Dr.Márquez No.162, Col. Doctores, Ciudad de México, México.*malvarez@himfg.edu.mx

Introduction. Medulloblastoma (MB) is the most common type of pediatric malignant brain tumor, accounting for about 20% of all childhood brain cancers. MB are heterogeneous group, the histological classification defines four variants, including classic, desmoplastic/nodular, anaplastic and large cell. These subgroups have different outcomes and recurrence patterns. Actual therapy consists of surgical resection, craniospinal irradiation, and chemotherapy, leading to 50-80% five years overall survival. However, long-term sequelae of patients treated for MB, including motor, sensory, endocrinological, cognitive and neuropsychological deficits, affect their quality of life.

Deregulation of microRNAs (miRNAs) expression play an important role in biology a wide variety of human cancers and proposed as targets for anticancer therapies. miRNAs are small noncoding RNAs with gene expression regulatory functions and are involved in the communication between cancer cells and the surrounding tumor microenvironment. The interaction between tumor cells and components of microenvironment may directly influence all aspects of cancer processes. Recent studies have shown that miRNAs released by cancer cells within microvesicles can reach and bind to Toll-like receptors (TLRs). Nevertheless the biological roles of miRNAs in medulloblastoma remain largely uncharacterized. The aim of this study is to determine the effect of miR-21 and let-7g on migration and invasion of medulloblastoma cells and role of TLR8.

Material and methods. The basal expression of TLR8 was determined by immunofluorescence, miR-21 and let-7g expression by real-time PCR in HTB-186 medulloblastoma cells. The cells were stimulated with the synthetic sequences of mature miR-21, let-7g or antagomiR-21, using Lipofectamine RNAiMAX. Stimulus times and concentrations were determined according to the needs of each assay. To evaluate the effect of miRNAs on cell migration, "wound" tests were performed in monolayer culture and for the invasion assay the HTB-186 cells were cultured in transwell plates with matrigel. The colocalization of the miRNAs with the TLR8 was done by confocal microscopy.

Results. We found that both miRNAs and TLR8 are expressed basally in HTB-186 cells. We observed that both miR-21 and let-7g affect the migration and invasion capacity of medulloblastoma cells. In addition, confocal microscopy assays showed that both miRNAs colocalize with TLR8.

Conclusion. The miR-21 and the let-7g affect characteristics that confer greater degree of malignancy to cells from human medulloblastoma, probably through a non-canonical pathway (as ligand of TLRs) and their study is a tool to establish therapeutic targets with great potential.

IMPLEMENTATION OF *EX VIVO* ELECTRORETINOGRAM (ERG) THE TECHNIQUE IN ISOLATED MOUSE RETINAS.

Margarita Marlene Araujo Meza., Ramses Noguez Imm., Ataúlfo Martínez Torres., and Stéphanie Thébault. Laboratorio de Neurobiología Molecular y Celular (D15), Instituto de Neurobiología, UNAM Campus Juriquilla, 76230 Querétaro, México.
(55) 56234064.
Correo del jefe de grupo: stephaniethebault@gmail.com.

Scientific research about diabetic mellitus and its microvascular complications, including diabetic macular edema and diabetic retinopathy, has led to the development of techniques that assess relevant functional activity of the retina to help predict a drug‟s efficacy. One such technique is the electroretinogram (ERG). ERG registers the electrical activity of the retina in response to light stimuli. In this work we implemented *ex vivo* electroretinogram (ERG) in mice because this configuration ensures that the activity arises from the retina and it also provides a direct access to the retina in comparison with *in vivo* ERG. We set-up a custom-designed specimen holder in combination with white light stimulation, heating unit, gravity-controlled perfusion system, and electromagnetic noise shielding to conduct simultaneous *ex vivo* ERG recordings from two isolated mouse retinas. So far, we have recorded *ex vivo* ERG using Ames and artificial cerebrospinal fluid solutions. Both conditions showed transretinal voltage fluctuations that are similar in nature with the *in vivo* ERG signal (a, b, and c-waves). We plan to test two additional physiological solutions, Locke and Ringer‟s, to determine which one provides the signal of major amplitude. Here, we used control male and female C57BL6/J mice and pretend to pursue this work using streptozotocin-treated mice as a model of type 1 diabetes, as well as *Trpv4*$^{+}$ mice, to compare the response between these models. In addition to the evoked ERG, we also aim to record basal ERG responses. **L**

Molecular and electrophysiological characterization of glutamate-gated chloride channels subunits from *Procambarus clarkii* crayfish's eyestalk.

Juan Manuel Arias Montaño[1]†, Itzel Scarlett Moreno Ramírez[1], Erick de Jesús Gómez Sánchez[2], Ubaldo García Hernández[2].
[1]Facultad de Estudios Superiores Iztacala, UNAM. Avenida de los Barrios 1, Col. Los Reyes Iztacala, Tlalnepantla, Estado de México, CP 54090.
[2]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional. Av. Instituto Politécnico Nacional 2508, Col. San Pedro Zacatenco, Gustavo A. Madero, Ciudad de México, CP 07360.
†Autor responsable; e-mail: jmarias@comunidad.unam.mx.

In vertebrates, neurotransmitter receptors from the Cys-loop family usually are hetero-pentameric assemblies arising from the combination of several dozens of subunits. Upon binding of its ligand, either GABA, glycine, acethylcholine or serotonin, receptors are activated to allow the ionic influxes of cations or anions, which in turn depolarizes or hyperpolarizes, respectively, the membrane in which they are expressed. In invertebrates, although the overall structure of these receptors is similar, they are gated by additional neurotransmitters, such as histamine and glutamate, and sometimes show different permeabilities when compared to its homologues in vertebrates.

Electrophysiological recordings from acutely isolated neurons from the crayfish X-organ, show responses to most of the above cited neurotransmitters, which evidence the presence of those receptors in these cells. Glutamate elicited currents showed transitory and sustained phases, which on average were 0.4± 0.05 nA with an EC_{50} of 0.07 mM and n_H = 3.88 (n = 14) for that agonist. Ionic substitutions indicate that both components are carried by chloride.

In order to identify the subunits assembling such receptors on these neurons, we took advantage on data available in nucleotide databases. Crayfish eyestalk transcriptome allowed us to design primers specific for transcripts that codify for peptides bearing high identity (50-80%) to subunits already reported in other species, like those from the nematode *C. elegans* and the fruit fly *Drosophila*. Amino acid sequence analysis of a cloned full-length cDNA shows the typical architecture of the Cys-loop receptor family members, i.e. the four trans-membrane (TM) domains, extracellular amino and carboxyl ends and three loops connecting the four TMs. Amino acids located on the selectivity filter predict an anionic permeability for this subunit. An additional partial transcript was identified and we are trying to amplify its entire codifying sequence. The already cloned subunit was transferred to a mammalian expression vector for its electrophysiological and pharmacological characterization once expressed in HEK-293 cells.

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Expression and phosphorylation of PEBP1 in early focal brain ischemia on rat hippocampus

Jorge Daniel Corzo Toledo[1], Hugo Sanchez Hernández[1,2], Luis Enrique Villafuerte Morquecho[3,4], Febe Elena Cázares Raga[1], Alma Ortíz Plata[4], Fidel de la Cruz Hernández Hernández[1].
[1]Departamento Infectómica y Patogénesis Molecular, Cinvestav-IPN, Av. Instituto Politécnico Nacional Núm. 2508, San Pedro Zacatenco, Ciudad de México 07360; [2]PEIB, Universidad Politécnica de Quintana Roo; [3]Fac. de Medicina, Universidad Autónoma de Nuevo León; [4]Lab. de Neuropatología Experimental, Instituto Nacional de Neurología y Neurocirugía MVS, Tlalpan, Ciudad de México 14269.
cruzcruz@cinvestav.mx

Introduction. The cerebrovascular disease is the third cause of morbimortality globally; it is classified as ischemic and hemorrhagic. The focal brain ischemia is defined as the decrease in blood flow in a specific brain area. The phosphatidylethanolamine-binding protein 1 (PEBP1) is a highly-expressed protein in the brain, it has a molecular weight between 17 to 23 KDa, and it is the precursor of the hippocampal cholinergic neurostimulating peptide. In stressful conditions the expression of PEBP1 decreases. The molecular mechanism of PEBP1 related to induced brain injury are currently under study. In this work, the expression and phosphorylation of PEBP1 was analyzed in the rat hippocampus by proteomic strategies during early focal brain ischemia. *Material and Methods*. Focal cerebral ischemia (I) was induced by transient middle cerebral artery occlusion (MCAO) on three months old male Wistar rats as experimental model. Times of 30, 60 and 90 minutes of ischemia followed by 24 h of reperfusion (R) after the surgery were used; intact animals (healthy) and only surgery (sham) controls were included. The subjects were sacrificed, the hippocampus was isolated and proteins were extracted.

Proteins were analyzed by SDS-PAGE and two-dimensional electrophoresis (2DE) and Western Blot (WB) was performed using heterologous anti-PEBP1 and antipPEBP1-S153 antibodies. Hippocampus coronal planes slices were used for immuno histochemistry (IHC) analysis by using the same antibodies. *Results*. In 2DE analysis several spots detected by antibodies probably corresponding to PEBP1 had changes in abundance among ischemia conditions; to validate the antibody detection a spot was studied by MS and identified as PEBP1**,** whose expression increased at 60 min with ischemia and decreased at 60 min with I/R. Total and phosphorylated PEBP1 species analyzed by WB, reached a peak at 60 min of ischemia, decreasing at 90 min with I/R. The behavior of the total and phosphorylated forms of PEBP1 was similar when analyzed by IHC. *Discussion*. The increase in the expression and phosphorylation of PEBP1 on the rat hippocampus during early ischemia and ischemia/reperfusión suggests that the protein could be involved in the activation and signaling related with protection mechanisms produced by the decrease on cerebral blood flow.

Effect of polypyrrole/iodine on GAP-43, GFAP and AKT expression in a model of chronic spinal cord injury in rats.

Angélica B. Coyoy Salgado, Carlos E. Orozco Barrios, Rodrigo Mondragón Lozano, Stephanie Sánchez Torres, Araceli Díaz Ruíz, Camilo Ríos, Guillermo J. Cruz, María G. Olayo, Roberto Olayo, Juan Morales, Omar Fabela Sánchez, Christian H. Guerra Araiza, Hermelinda Salgado Ceballos.
CONACyT- IMSS, C.P. 06703; Proyecto Camina A.C, C.P. 14050
Tel. 56276900 Ext. 21367
Email: acoyoys@gmail.com

Spinal cord injury (SCI) is an overwhelming neurological disorder that affects approximately 180 000 new individuals each year and a total of 1.3 million persons worldwide. Causes include vehicle accidents, violence, accidental falls, and other traumatic events. SCI results in an initial mechanical disruption of structures in the spinal cord, which is called the primary insult. There after, SCI leads to a cascade of secondary events that collectively injure the intact neighboring tissue. This secondary injury includes: blood–brain barrier dysfunction and thrombosis resulting in edema and ischemia, free radical formation, gliosis and increased glutamate release that leads to neuronal death.
Many researchs has worked for functional recovery in acute to subacute phase spinal cord injury (SCI). Our research group has used polypyrrole iodine doped ((PPy/I) polymer synthesized by plasma for implantation into the contused spinal cord tissue, where we showed that polymer is biocompatible and is able to reduce the inflammatory response, increase tissue preservation and improve functional recovery after SCI in adult rats (Alvarez- Mejia 2015). By contrast, there are still only a few studies have focused on the treatment of the chronically injured spinal cord, in which the treatmens seems less effective. Since the majority of SCI patients are in the chronic phase.
In the present study, we evaluated the effect of PPy/I in a model of chronic spinal cord injury on some oxidative stress markers, like 4-HNE and protein nitrosylation; the protein expression of glial fibrillary acidic protein (GFAP) that is a protein of mature astrocytes and was used as a marker of reactive astrogliosis, and also to determinate axonal regeneration we evaluated the protein expression of growth-associated protein-43 (GAP-43). Furthermore we evaluated the protein levels of Akt kinase and phosphorylated Akt, this enzime plays a crucial role regulating neuronal proliferation, differentiation, and survival; the phosphorylated Akt can protect cells from apoptosis via stimulation of the expression of proteins that favor cell survival and by inhibiting executor caspases.
The PPy/I was implanted 30 days after a SCI produced by contusion, after 1 month the rats was sacrificed and dissected the spinal cord at T9. Through the western blot technique we found that the PPy / I reduced the oxidative stress, decreased the expression of GFAP, but did not modify the expression of GAP-43 or phosphorylated AKT.
In conclusion, PPy/I in a chronic spinal cord injury in rats did not favor axonal regeneration, but oxidative stress and gliosis decreased, which could favor motor recovery in this lesion.

HISTOLOGICAL CHARACTERIZATION INVOLVED IN PATHOPHYSIOLOGY OF THE SPINAL CORD INJURY IN DIABETIC RATS

Adriana Domínguez-Vázquez[1]., Fernanda Tenorio-Cano[1], Jimena Otero-Negrete[1]., Pedro Medina-Granados[2]., Cynthia Sámano-Salazar.[1]*
[1]Depto. de Ciencias Naturales e Ingeniería, UAM-Cuajimalpa
[2]Depto. de Biología Celular y Fisiología, Instituto de Investigaciones Biomédicas, UNAM
*Avenida Vasco de Quiroga 4871, Col. Santa Fe Cuajimalpa. Delegación Cuajimalpa de Morelos, C.P. 05348, Ciudad de México. e-mail: cynthia.samano@gmail.com

The spinal cord injury (SCI) whether traumatic or non-traumatic, often results in severe dysfunction and disability because is an incurable situation with a high incidence of morbidity and mortality, affecting each year millions of people. The SCI is a very complex and devasting mechanism which goes through two critical steps: *(i)* the primary injury, which is the initial trauma, and *(ii)* the secondary injury, when the damage considerable expands due to further destruction of neural and glial cells caused by post-traumatic inflammatory reactions, metabolic dysfunction and excitotoxicity. Few studies have examined the mechanisms by which SCI could be aggravated when coincides with a metabolic disorder, like Diabetes Mellitus (DM). Previous studies have shown that DM exacerbates the vulnerability to traumatic injury and limits the functional improvement after SCI in animals and humans. Thus, we decided to identify the specific regions in the grey matter (GM) and white matter (WM) with more susceptibility to SCI and DM. In this way, our model consisted in injected streptozotocin (STZ) to induce type-1-like DM in the Wistar adult rats, and the injury was induced by compressing the spinal tissue with a vascular clip at the lumbar level (L1-L2, where the main components of the locomotor Central Pattern Generator are located). The experimental groups were: sham (untreated animals), diabetes, lesion and diabetes + lesion (D+L), and the rats were perfused 4, 48 or 72h post-injury. The samples were processed for immunohistochemistry to detect NeuN (specific nuclear protein) cells positive in the GM, GFAP (specific intermediate filament of astrocytes) fibers positive in the WM, and pyknotic nuclei with DAPI staining. We found and evident reduction in the number of NeuN cells positive after lesion condition in central and ventral regions, while all regions of the GM were affected after diabetes and D+L. However, the reduction of neurons was significantly lower in D+L condition, versus only diabetes. Analyzing the GFAP staining, we found that after lesion condition the fibers reduced significantly in the WM regions, but significantly increased in the GM. In the case of diabetes and D+L there was a notably reactive gliosis in both GM and WM, nevertheless it is important to mention that those fibers were morphologically different versus sham condition. These results did not vary for each condition at different post-injury time. Additionally, we explored the staining to SIRT1 (NAD^+-dependent deacetylase, related with neurodegenerative process) after each condition, and we found that it is expressed in the neurons of the GM only 4h post-injury, indicating SIRT1 expression is limited to the chronic stage. Although more studies need to be done, we can mention that DM amplifies the SCI in a specific region in GM and WM. It is possible that the early expression of SIRT1 is necessary to activate to subsequent cellular processes required to counteract the damage, however this need to be investigate.

P2X3 receptors participate in neuronal hyperexcitability during acute colitis

Ismael Eduardo Gallegos Vieyra, Felipe Barrera Patiño, Diana Alvarado Jiménez, Tonatiuh Barrios García, Eduardo E. Valdez Morales, *Raquel Guerrero Alba
Universidad Autónoma de Aguascalientes, Centro de Ciencias Básica, Departamento de Fisiología y Farmacología. Av. Universidad # 940. Colonia Ciudad Universitaria. C.P. 20130. Tel (449) 9 10 7400 ext 51261 o 8407
*correo electrónico raquel.guerrero@edu.uaa.mx

Background and aim: Previous studies have been shown that acute inflammation in the colon enhances excitability of colonic nociceptive dorsal root ganglia (DRG) neurons[1] but the molecular mechanisms involved in this hyperexcitability is not fully understood. Extracellular adenosine 5‟-triphospate (ATP) has been established as a key sensory signaling molecule that produce more pronounced nociceptive response after inflammation[2]. Extracellular ATP exerts its effects through P2 receptors, some of which are ligand-gated ion channels, called P2X receptors. At present, seven functional P2X receptors ($P2X_1$-$P2X_7$) have been identified, of which the P2X3 receptors is highly expressed in nociceptive DRG neurons[3]. We don‟t know if P2X3 receptor is involved in the hyperexcitability of DRG neurons caused during acute inflammation. Therefore, the aim of the present study is to determine if P2X3 receptor participate in neuronal hyperexcitability during an inflammatory process.

Methods: Colitis was induced by intrarectal administration of Dinitrobenzene sulfonic acid (DNBS) 200 mg/kg in ethanol 50% in C57BL/6 mice. The severity of colitis was assessed by weight loss and macroscopic and microscopic scores of damaged colonic tissues. To evaluate the neuronal excitability, T8-L2 Dorsal Root Ganglia (DRG) neurons were dissociated from control and DNBS mice and then changes in rheobase and action potential firing were measure using perforated patch clamp technique. The role of P2X3 receptor was studied using a selective P2X3 antagonist A-31749, in cultured DRG neurons incubated with tumor necrosis factor alpha (TNF-α), a key mediator of the pronociceptive effect during ulcerative colitis4.

Results: Control mice continued gaining weight over the 4 days study, whereas, DNBS-treated mice lost weight significantly by 48 hr. Colonic sections collected 4 days post DNBS treatment showed focal ulceration, crypt destruction, goblet cell depletion and mucosal infiltration of immune cells. DRG neurons from DNBS-treated mice showed increased excitability (rheobase decreased 51%, $P < 0.05$, unpaired *t*-student test). However, the action potentials discharges at two rheobase wasn‟t statistically significant. Incubation of DRG neurons with TNF-α recapitulated the hyperexcitability effect of DNBS-treated mice on rheobase and this effect was blocked with the selective P2X3 antagonist receptor (A-31749).

Conclusions and inferences: These results suggest that the release of proinflammatory mediators during colitis, such as TNF-α, enhanced excitability of sensory DRG neurons innervating the colon through of modulation of activity P2X3 receptors.

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Participation of $GABA_A$ receptors in the antidepressant-like effect of *Justicia spicigera* Schltdl in female rats in the metestrus-diestrus phases of the ovarian cycle in Wistar rats.

Rosa Isela García-Ríos [1], Armando Mora-Pérez[1], Cesar Soria-Fregozo [1], Ricardo de Jesús Carpio Reyes[1], Cristian Fabián Enríquez López[1].
[1]Laboratorio de Ciencias Biomédicas/Área Psicobiología e Histología. Centro Universitario de los Lagos de la Universidad de Guadalajara. Av. Enrique Díaz de León N° 1144, Col. Paseos de la Montaña, C.P. 47460, Lagos de Moreno, Jalisco, México. Teléfono: +52 (474) 742 4314, 742 3678 Fax Ext. 66527. Coreo:rosai_garios@yahoo.com

Background: The infusion of the *Justicia spicigera* leaves, a plant known as "muicle" in traditional Mexican medicine have been used as nervous tonic, a blood tonic, to regulate menstrual impairments and to treat sadness. The infusion of *Justicia spicigera* leaves (IJsL), in the doses used for treatment of sadness in humans, 24 mg/kg, exerts an antidepressant-like effect, in the metestrus-diestrus phases of the ovarian cycle, after 21 days of treatment and similar to the clinically effective antidepressant as fluoxetine. The presence of terpenes, flavonoids and sterols in the IJsL were confirmed. This metabolites group increased serotonin and norepinephrine in mouse and rat brain. Additionally, flavonoids have demonstrated anxiolytic and antidepressant effects through the modulation of $GABA_A$ receptor. However, it is currently the mechanisms of action through the IJsL exerts its effects are still unexplored.

Objective: To explore the participation of $GABA_A$ receptor in the effects produced by the treatments, through their antagonism in the antidepressant-like effect of the IJsL, in metestrus-diestrus phases of the ovarian cycle in Wistar rats subjected to locomotor activity and forced swim test.

Methods: We used 4 experimental groups (n=10/group) that received: vehicle, purified water 1.8 ml/kg, v.o (VEH); 24 mg/kg of the IJsL, v.o. (24 mg/kg IJsL); VEH v.o. + picrotoxin (noncompetitive $GABA_A$ chloride channel antagonist picrotoxin) 0.5 mg/kg, i.p. (VEH + PTX); picrotoxin 0.5 mg/kg, i.p. + 24 mg/kg of the IJsL, v.o. (PTX + 24 mg/kg IJsL). The v.o. treatments were administered for 21 consecutive days, while PTX i.p. was administered 30 minutes before the last oral administration and 90 minutes before the test of locomotor activity and forced swim test.

Results: The latency to the first immobility were not significant modified by treatments (p=0.130). However, the reduction in immobility caused by the administration of 24 mg/kg of the IJsL was replicated, effect that was partially reversed with the previous administration of PTX (p<0.001). In addition, these effects are not associated with significant changes in the crossing (p=0.523) with any of the treatments evaluated.

Conclusions: The $GABA_A$ receptors partially participate in the modulation of the antidepressant-like effect of the infusion of *Justicia spicigera* leaves in the metestrus-diestrus phases of the ovarian cycle in Wistar rats. Effect could be due to the interaction between flavonoid terpenes and sterols contained in the infusión and the $GABA_A$ receptors.

Role of Wnt signaling pathway on hippocampal reorganization after a synaptic loss

Lizbeth E García-Velázquez,[1] Clorinda Arias.[1]
[1] Department of Genomic Medicine and Environmental Toxicology, IIB, UNAM.
Circuito exterior, Ciudad Universitaria. CP. 04510
Tel. (+52)55 56229215 Mail: lizgarvel@comunidad.unam.mx

Neuronal plasticity is fundamental for structural and functional adaptations of neuronal circuits. The remodeling of spines and axons in a dynamic way helps neurons to maintain and reestablish homeostasis in the circuit after the loss of synapse, which may be due to a lesion or to a variety of neurological diseases. Several molecules have been proposed to favor synaptic plasticity, including neurotrophins and cellular adhesion molecules. However, Wnt signaling pathway components could also display a significant role in these processes and have been scarcely explored in this sense. It has been shown that Wnt5a and Wnt7a ligands are expressed in the adult brain and favor the assembly of pre- and post-synaptic clusters. Furthermore, these ligands also increase the number and size of dendritic spines and promote the growth of the neuronal cone in vitro. Nevertheless, it remains unknown if Wnt signaling pathway is activated in response to a synaptic loss and participates in the synaptic reorganization after damage.

In this work, we have evaluated changes in the expression and function of Wnt agonists (Wnt5a, Wnt7a) and antagonists (Dkk1 and Sfrp1) in a model of hippocampal deafferentation. Male Wistar rats of 250-300g were used throughout the study. Unilateral injection of kainic acid (1µL, 2µM) was applied to the right medial entorhinal cortex (EC) (A=27.64, L= 25.4 and V= 25.6). Animals were sacrificed by decapitation 1, 3, 7 and 30 days after lesion and the right hippocampus was dissected. Control animals received 1µL of 10 mM phosphate buffer. Total RNA and protein were obtained using TRizol. Expression analysis of *Wnt5a*, *Wnt7a*, *Sfrp1* and *Dkk1* were carried out by qRT-PCR. Protein levels of Wnt5a, Wnt7a, Sfrp1, Dkk1, CycD1, c-Myc, p-MAP2 and ABC were analyzed by Western Blot. Structural analysis was assessed using AChE, Fluorojade and Nissl stain protocols.

We have found a differential expression of the analyzed genes along time; an increase in the *Wnt5a* transcript levels was observed during the first 72h, whereas a gradual increase in the levels of *Wnt7a* mRNA was evident along time. The active form of β-catenin, a downstream protein of the canonical Wnt pathway, increases after the lesion, getting its highest levels from the seventh day. This suggests the activation of the canonical pathway, which is supported by the increase of Cyclin D1 protein, a downstream target of β-catenin. Interestingly, an increase of the p-MAP2C protein, an isoform related to dendrite outgrowth, was observed during the same period. All these processes are accompanied by changes in the hippocampal structure along time.

These results suggest the activation of the Wnt canonical pathway in response to the synapse loss, which could contribute to the structural reorganization of the hippocampus after its deafferentation. Wnt7a ligand may have a key role as an inducer for the activation of the Wnt canonical pathway under these conditions, however, this may be proven in future experiments.

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Pharmacological characterization of the heterologous expression of P2X1 and P2X1 *del* human receptors

Karen Sarahí Gómez Coronado, Rosa Espinosa Luna, Alma Rosa Barajas Espinosa, Carlos Barajas López.
Instituto Potosino de Investigación Científica y Tecnológica, A. C. (IPICYT)
Camino a La Presa San José 2055, Lomas 4ta sección, C.P. 78216, San Luis Potosí, S.L.P., México. Tel. (444) 834 20 32
karen.gomez@ipicyt.edu.mx

Abstract

Human leukocytes release nucleotides, including ATP, bind to purinergic receptors to trigger a number of cellular process, such as the death of pathogens.The understanding of the immunomodulator mechanisms of nucleotides could help to develop new therapeutic strategies for immune disease and inflammation.Currently, there are few studies on the human P2X1 receptor (hP2X1) and its splicing variant (hP2X1 *del*), previously reported as non-functional. Our research group was the first to describe this splicing variant in human monocytes and since little is known about the pharmacological properties of hP2X1 and its splicing variant hP2X1 *del* , aim here was to characterize these properties.

In this study, we measured the currents induced by ATP using the two-electrodes voltaje clamp technique, in oocytes of *Xenopus laevis* injected with hP2X1and P2X1 *del m* RNA. ATP concentrations ranged from 0.1 to 5000 µM for hP2X1 and 100 to 5000 µM of ATP for hP2X1 *del* . Our showed that currents mediated by the hP2X1 receptors have two phases and their activation and inactivation kinetics are independent of each other. At low concentrations of ATP (0.1-300 µM) the hP2X1 receptor shows a high sensitivity (EC50 = 2.72 µM) to ATP and rapid desensitization, however at high concentrations of ATP (3000 and 5000 µM) applied for 1 min the hP2X1 receptor presents a rapid desensitization response and a second response that slowly increases with the time of exposure to ATP. For the hP2X1 *del* receptor ATP concentrations of 300 µM, 1000 µM, 3000 µM and 5000 µM were applied for 30 s, currents were generated at 3000 µM and 5000 µM that increase with the time of exposure to ATP, effect similar to the second phase of the hP2X1 receptor at high concentrations of ATP.

One of the two binding sites has high sensitivity to ATP and mediates the rapid inward current, which decreased in only few seconds, in despite the continuous presence of the agonist. The second binding site has a low sensitivity to ATP, causing a second phase, which amplitude increases slowly as a function of ATP exposure time.

Loss of the autophagic flux in the brain of old rats favors the establishment of cellular senescence

Elisa Gorostieta-Salas[1], Daniel Moreno-Blas[1] and Susana Castro-Obregón[1]
[1] Institute of Cellular Physiology, Department of Neurodevelopment and Physiology
National Autonomous University of Mexico, C.P: 04510
Tel: 56225676 email: scastro@ifc.una.mx

Aging is defined as the progressive loss of physiological functions of the organism and an increased vulnerability to die. Loss of proteostasis, cellular senescence, genomic instability and mitochondrial dysfunction are some of the hallmarks of aging. Loss of proteotasis is of particular importance because it ensures protein integrity in order to maintain the health of cells and the organism itself. Autophagy is a catabolic process that regulates the degradation of intracellular material like misfolded proteins and damaged organelles, contributing to maintain proteostasis. During aging, loss of autophagic activity and the increase of ubiquitin-positive protein aggregates are observed in various tissues, including the brain. Experimental strategies that activate autophagic flux also increase the lifespan in different organisms, therefore we propose that aging is at some level a consequence of autophagy dysfunction.

Even though cellular senescence has physiological functions, their accumulation during aging is harmful to the organism as they alter the surrounding tissue by promoting local inflammation; they also induce paracrine senescence, spreading the senescent phenotype along old tissues. At least in mice, persistent senescent cells contribute to aging and age-related diseases; therefore, it is fundamental to find strategies to prevent cellular senescence establishment and maintenance. Senescent cells are characterized by an increase of senescence-associated β-galactosidase activity, accumulation of DNA damage and activation of the DNA damage response, accumulation of lipofuscin, and increased expression of the cell cycle inhibitors $p21^{CIP1}$ and $p16^{Ink4}$, among other features. Neurons with those senescent features have been observed in old mice brains. We observed that also in old rat brains senescent cells accumulate and the function of autophagy is impaired particularly in the hippocampus. We propose that loss of autophagic function promotes both neuronal and glial senescence in this brain area, leading to cognitive decline. To test this hypothesis we induced autophagy in old rats during 2 months and evaluated whether the number of senescent cells decreased in the hippocampus. We will discuss our findings as well as the potential improvement of cognitive performance in old animals.

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Differences in the plasma concentration of lipids and β-amyloid and tau protein between Mexican patients with Alzheimer's disease and healthy subjects

Christian Guerra-Araiza[1], Tzayaka Castillo-Mendieta[1], Julia Segura-Uribe[2], Juan Manuel Gallardo[3], Victoria Campos-Peña[4], Marvin Soriano-Ursúa[5].

[1]Unidad de investigación Médica en Farmacología, [2]Unidad de Investigación Médica en Enfermedades Neurológicas, [3]Unidad de Investigación Médica en Enfermedades Nefrológicas, CMN S XXI, IMSS; [4]Instituto Nacional de Neurología y Neurocirugía, Secretaría de Salud, [5]Escuela Superior de Medicina, Instituto Politécnico Nacional
Ciudad de México, México.
Av. Cuauhtémoc 330, Col. Doctores, Del. Cuauhtémoc. C.P. 06720. Ciudad de México. Tel. 56276900 ext. 21367. *E-mail*: christianguerra2001@gmail.com

Alzheimer‟s disease (AD) is a neurodegenerative disorder classified as the most common of the dementias. Currently, no clinical, laboratory, genetic or neuroimaging research can provide an early diagnosis. Moreover, the confirmation of AD diagnosis can only be performed with a post-mortem brain examination. Therefore, a non-invasive method for early diagnosis is necessary. A group of Mexican patients > 60 years of age clinically diagnosed with AD (n = 27) and control subjects without any associated neurological diseases (n = 20) were analyzed to determine the concentration of the β-amyloid and tau protein in plasma. Samples were processed by Enzyme ImmunoAssay (ELISA) adsorption for β-amyloid 40 and total human tau.

Our findings showed that the concentration of β-amyloid 40 was higher in the plasma of patients with AD when compared with the control group, while the concentration of total tau protein was similar in both groups. Cholesterol and triglycerides levels were also analyzed. In the case of cholesterol, the concentration was similar in both groups, whereas a significant increase in the concentration of triglycerides was observed in plasma of patients with AD when compared with healthy subjects.

These results suggest that the concentration of the protein β-amyloid and triglycerides can be used as a plasma marker for AD early diagnosis in Mexican patients.

Integrating genomic and metabolomic data to elucidate the biochemical mechanisms related to schizophrenia

Nora Gutiérrez-Nájera[1], Ángel Polanco[2], José Juan Ordaz[3], Mirna Morales-Marín[1], Alma Genis[3], Jaime Martínez Magaña[2], Xochilt Castro[1], Osbaldo Resendis[4], Claudia Erika Hernández Patiño[2], Christian Diener[1], Juan Carlos Fernández[1], Humberto Nicolini[1].
[1].- Instituto Nacional de Medicina Genómica, SS; [2].- Universidad Nacional Autónoma de México; [3].- LANGEBIO CINVESTAV Irapuato; [4].- Coordinación de la Investigación Científica, Red de Apoyo a la Investigación, UNAM/INMEGEN.
Instituto Nacional de Medicina Genómica, Periférico Sur No. 4809, Col. Arenal-Tepepan, CP 14610, Tlalpan, Ciudad de México. E-mail: ngutierrez@inmegen.gob.mx; hnicolini@inmegen.gob.mx.
Tel. 5350-1900 ext. 1218

The psychiatric disorders as schizophrenia are complex diseases. The combination of genomic information together with a detailed molecular analysis of the samples of the patients will be important to predict, diagnose and treat psychiatric diseases. Metabolomics provides an approach to understand the biochemical regulation of metabolism and networks in a biological system. The objective of this work was to discover new mechanisms and biomarkers for psychiatric disease studying the composition of metabolites in urine from patients with psychiatric diseases, whose were previously genotyped with the Infinium PsychArray Bead Chip of ILLUMINA®, integrating the metabolomic and genomic data by a Systems Biology approach.The study used samples from 37 patients with schizophrenia and control samples (24) from donors without psychiatric disease. We studied the composition of the metabolites in urine of healthy individuals and patients with psychiatric disease. The urine samples were analyzed by liquid chromatography- mass spectrometry. The blood samples also were genotyped with the Infinium PsychArray BeadChip Illumina® which allowed obtention of genomic data. Data analysis combined genomic and metabolomic data by using a bioinformatic approach of the Systems Biology using the Progenesis, MetabolAnalyst and Reactome softwares and KEGG, MassBank, METLIN and Human metabolome databases. The data led to identify metabolites as glycocholic acid as associated to schizophrenia. Currently we are analyzing the relation of the levels of this metabolite with single nucleotide polymorphisms of the genes involved in the pathway for the primary bile acid biosynthesis, in order to integrate a mechanism to propose targets as biomarkers for psychiatric diagnosis of schizophrenia.

Study of neural differentiation *in vitro* using immortalized multipotent otic progenitor cells.

Edson Daniel Hernández Velázquez, Víctor Hugo Hernández González, PhD. MD; Valeria Piazza, PhD. Division of Sciences and Engineering, University of Guanajuato/Optics Research Center "CIO".
Loma del bosque 103, León, GTO., 37150. México, Tel: +52 (477) 7885100 x 8406. Email: vh.hernandez@ugto.mx

Sensorineural deafness is a type of hearing loss due to damage in the inner ear, mainly secondary to the use of some drugs, noise exposure or genetic mutations. When this happens, cochlear implants can be useful to enable hearing comprehension.

Cochlear prosthetics are among the most successful neuroprostheses, however for a good performance they need to find the spiral ganglion neurons (SGN) in good state, which is not always possible. Damage to SGN is irreversible as they are postmitotic. Many efforts have been done to block neural degeneration or even regenerate these cells.

In this work, we use an immortalized multipotent otic progenitor cell line (IMOP), to induce its differentiation into SGN. Our objective is to characterize the process of differentiation from the acquisition of neural identity to the display of mature otic features.

The IMOP cell line was obtained rom the inner ear of mouse, and it can be differentiated into neurons using a specific combination of growth factors and media. After consistently obtaining the IMOPs derived "neurons" we characterized them using immunostaining, to determine characteristic features of their morphology and their identity, for example by testing different substrates for optimal differentiation of the cells.

We have also worked on the estimation of the lifespan of the neurons in *in vitro* conditions using vital dyes. These findings could allow the design of cell therapy strategies for the cases where damage of SGN is involved in the hearing loss.

Evaluation of the anti-inflammatory and antioxidant activities of scammonin I isolated from *Ipomoea tyrianthina* root.

Johanna Stefan Jaimes Acuña, José Manuel Castro García, María del Carmen Gutiérrez Villafuerte*
Centro de Investigación en Biotecnología (CEIB), Universidad Autónoma del Estado de Morelos. Av. Universidad 1001 Col. Chamilpa C.P. 62209. Cuernavaca, Morelos. Tel: 01 (777) 3297057 Ext. 4556 y Fax: (777) 3297030 E-mail: carmengu@uaem.mx

Neuroinflammation and oxidative stress are constituent of neurodegenerative diseases characterized by the release of pro-inflammatory cytokines and reactive oxygen species (ROS), generating neuronal damage and even death [1]. Recently, extracts and metabolites obtained from glycosidic resins produced by *Ipomoea tyrianthina* Lindley have demonstrated activity on the central nervous system, especially related to the GABAergic system (2), such as: sedative, antidepressant, anticonvulsant and / or neuroprotective activities. Since scammonin I obtained from extracts of *Ipomoea tyrianthina* would eventually be considered as a potential drug, the objective of this project was to evaluate its neuroprotective activity in mice in an immunohistochemical *in vivo* study and *in vitro* study. *I. tyrianthina* (1: glycolipid scammonin I, 2: aromatic compound) isolated from the root of *Ipomoea tyrianthina* Lindley was used in this study.

***In vivo* study:** The effect of three concentrations of scammonin I (40, 80 and 120 mg/kg) in mice treated with pentylenetetrazole (PTZ) was evaluated to determine the expression of pro-inflammatory cytokines (IL-1β,IL-6 and TNF-α) in three sections of brain tissue (cortex, hippocampus and cerebellum). At the concentration of 120 mg / kg scammonin had a neurotoxic effect: cells were reactive at the three cytokines; in addition, pyknotic cells and interstitial edema were observed, similar to mice treated with PTZ alone. The major damage was observed in the hippocampus, mainly in dentate gyrus, CA1 and CA3. The co-treatment of PTZ and scammonin I (40 mg/Kg) presented a favorable response against the agent of damage: a greater number of cells with defined core, less reactive cells to interleukin antibodies and a decreased in interstitial edema; this effect was observed in the three sections of the hippocampus.

***In vitro* study:** Assays were carried out to determine the capture capacity of the DPPH radical by scammonin I; the results demonstrated that scammonin I does not present antioxidant activity when using concentrations from 40 to 1280 µg/mL; the percentage of inhibition of free radicals (I%) maximum was I% = 1.60. These results were compared with the antioxidant ascorbic acid which presented an I% = 52.02 at a concentration of 40 µg/mL. Finally, the release of pro-inflammatory mediators in mouse brains will be carried out in a lipopolysaccharides model to quantify the cytokines IL-1β,IL-6 and TNF-α, as well as the quantification of reactive species through the lipid peroxidation technique.

In conclusions scammonin I decreases interstitial edema and the expression of pro-inflammatory interlucins (IL1-β, IL-6 and TNF-α). However, it does not have the capacity to donate electrons or H+ to free radicals.

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Regulation of TRPV1 channel by endocannabinoids

Rebeca Juárez-Contreras, Sara L. Morales-Lázaro
Departamento de Neurociencia Cognitiva, Instituto de Fisiología Celular, UNAM.
Circuito Exterior s/n, Ciudad Universitaria, México, D.F. 04510 México.
saraluzm@ifc.unam.mx

The vanilloid Receptor 1 (TRPV1) is an ion channel widely associate to chronic pain conditions as diabetic neuropathy, migraine, bowel irritable syndrome, temporomandibular joint and arthritis. This channel isactivated bytemperatures ≥ 43°C,chemical compounds, extracellular acid and intracellular basic pH.

Interestingly, the TRPV1 activation by capsaicin (a chemical compound found inhot chili peppers)increases the intracellular calcium concentration and triggers calcium-dependent „defunctionalization" of nociceptor fibres, the physiological consequence is a pain relief. For these reason, there are some patches or creams with capsaicincommercial available. However, capsaicin topical application produces collateral effects as extreme irritation and burning on the affected zone.

Until now, there are no evidences about the role of endogenous TRPV1 activators as compounds producing this type of analgesia. A putative molecule to produce similar analgesic effects as capsaicinis the endocannabinoid anandamide (AEA), which isa ligandof the cannabinoids receptors (CB1 and CB2)and alsothis is a partial agonist of the TRPV1 channel.

To describe and understand the possible regulatory role of AEA onTRPV1 pain regulation, we determined if AEA is able to produce similar effects as was previously reported by capsaicin, as internalization, lysosomal degradation and downregulation of TRPV1protein.

First, we found downregulation of the TRPV1 protein levels from DRG neurons treated with AEA. Second, we observed the same effect on HEK293 cells with transiently TRPV1 expression and treated with different AEA concentrations.Finally, we found that AEA decrease the pain response to capsaicin, suggesting that AEA has an important role to inhibit the pain associate to TRPV1 activation.

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Characterization of corticospinal neurons from the primary motor cortex in Alzheimer's model

Martín Macías, Erika María Orta Salazar, Rafael Olivares Moreno, José Gerardo Rojas Piloni and Sofía Yolanda Díaz Miranda (Hom. Sofía Díaz Cintra).
Developmental Neurobiology and Neurophysiology, Institute of Neurobiology, National Autonomous University of México, Querétaro, México; Boulevard Juriquilla #3001, Querétaro, 76230, México.
(55) 5623 4058, yoldi@unam.mx

Alzheimer"s disease (AD) is the most common type of dementia and is characterized by cognitive deficits accompanied with motor deficits. Motor control is related with movement execution and involves corticospinal neurons (CSp), which are located in layer V of the primary motor cortex (M1) and innervate the spinal cord. In this work, we investigate if some changes in this CSp neurons are present due to AD, using the mouse model (3xTg-AD), associated to its motor behavior. Thus, transgenic (Tg) and Non-transgenic (Non-Tg) female mice 11 and 16 months old (mo) were submitted to open field test for evaluate the spontaneous locomotor activity levels. Mice were injected with a neuronal tracer (Fluorogold) into the dorsal horn of the cervical C4-C5 spinal cord segments to difference CSp neurons. Five days post-injection, brains and spinal cords were obtained for histological processing. In order to evaluated the changes due to AD on CSp neurons localized on M1, sagittal brain slices of 50 micron"s thickness were stained with fluorescent Nissl (NeuroTrace). Results show that in the open field test, the rest time by Tg group 16 mo is significantly higher when is compared with Tg group 11 mo. In relation with the Non-Tg mice 16 and 11 mo, this paramenter is similar. However, Non-Tg mice 16 mo show a tendency to spend less time moving compared to Non-Tg mice 11 mo. In the morphological quantification we found that the percentage of the fluorescence-labeled CSp neurons in layer V of M1 is similar between Tg and Non-Tg female mice in both ages. In sum, Tg and Non-Tg female mice 16 mo appear to display signs of motor function deficits but no difference in the percentage of CSp neurons in none of the groups. Thus, changes in CSp neurons of M1, probably due to AD, will be evaluated as well as in males in order to make comparisons of sexual dimorphism and between ages in the 3xTg-AD model.
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Prolactin Protection Against Oxidative And Hypoxic Stress In Hippocampal Neurons

Fernando Macías[1], Miriam Ulloa[1], Josué Rivera[1] Rodrigo Manuel Aroña[1], Carmen Clapp[1], Gonzalo Martínez de la Escalera [1], Edith Arnold[1,2].
[1]Department of Celular and Molecular Neurobiology. [2]CONACYT Catedrática
Neurobiology Institute, Universidad Nacional Autónoma de México, Campus UNAM Juriquilla, Boulevard Juriquilla #3001, Juriquilla, 76230, Querétaro, Qro. México. (55) 5623 4028, earnoldh@gmail.com.

Oxidative stress (OS) is an imbalance in favor of pro-oxidants, like reactive oxygen species (ROS), against antioxidants. This process is commonly associated with a disruption in the redox machinery of the cell and with macromolecular damage. OS is related with the occurrence and progression of neurodegenerative diseases such as Alzheimer´s disease, Parkinson´s disease, Huntington´s disease and hypoxic-ischemic injury. Thus, efforts have been done to find antioxidant-protective factors that could diminish the cytotoxic effect of ROS. Prolactin (PRL) is a pituitary hormone, mainly known for its actions in lactation and reproduction. In the brain PRL acts as a neuroprotective factor. Some studies have reported that PRL protects hippocampal cells against excitotoxicity *in vitro* and *in vivo.* Furthermore, PRL protects cortical astrocytes and retinal pigment epithelium cells against oxidative damage induced by hydrogen peroxide (H_2O_2). Therefore, in this work we investigated whether PRL protects hippocampal neurons under OS conditions induced by H_2O_2 and hypoxic stress induced by cobalt chloride ($CoCl_2$). Primary cultures of hippocampal neurons were isolated from the brain of E16 mice and incubated for during 12 days *in vitro* (DIV). The cultures were characterized by immunocytochemistry for the expression of the specific neuronal marker β-III-tubulin. Purity and maturity of neuronal cultures was assesed by the expression of GFAP (astrocyte marker); sinaptophysin, vGlut1 and PSD95 (synapsis markers); and PRL receptor at DIV 4, 10 and 12 by qRT-PCR. The highest expression levels of the synaptic markers as well as the expression of the PRL receptor were observed at DIV10. At this time the cultures were incubated with increasing concentrations of H_2O_2 (25 to 225 M) or $CoCl_2$ (100 to 600 M), to determine LD50.

Some cultures were incubated with increasing concentrations of PRL (1 nM to 100 nM) at DIV9, before the challenge with H_2O_2 or $CoCl_2$ (LD50). The treatment with 50 nM and 100 nM of PRL prevents the reduction of the cell viability induced by H_2O_2 but not by $CoCl_2$, as determined by the MTT assay. These results suggest that PRL is a potential neuroprotective factor against oxidative stress.

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Malva parviflora extract regulates the phagocytic capacity of microglial cells via a PPARγ-mediated mechanism in an Alzheimer´s disease model

Elisa Medrano-Jiménez[1], Martha Pedraza-Escalona[1], Lourdes Álvarez-Arellano[1], Alejandro Zamilpa-Alvarez[2], Maribel Herrera-Ruíz[2], Enrique Jiménez-Ferrer[2], Jaime Tortoriello[2], Gustavo Pedraza-Alva[1] and Leonor Pérez-Martínez[1]

[1]Laboratorio de Neuroinmunobiología, Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología, Universidad Nacional Autónoma de México (UNAM), Cuernavaca, Morelos 62210, México. [2]Centro de Investigación Biomédica del Sur, Instituto Mexicano del Seguro Social (IMSS), Argentina No. 1, Xochitepec, Morelos CP 62790, México. Correspondence: leonor@ibt.unam.mx

Microglia are immune cells of the CNS that participate both in normal CNS function and in disease. Recent evidences indicate a role for activated microglia in the Alzheimer"s disease (AD) progression since they release pro-inflammatory cytokines that induce neuroinflammation, which compromises microglial clearance functions. These observations have proposed to the microglia as a therapeutic target for AD treatment. Agonists of PPARγ display anti-inflammatory properties and stimulate microglial Aβ phagocytosis via a CD36 dependent mechanism. Nevertheless, the side effects produced by these agonists limit their use. Natural agents have been widely used for long time for treating diseases with an inflammatory origin. Recent studies have shown that *Malva parviflora* extract has anti-inflammatory, hypoglycemic and anti-oxidant activities. Here we investigated the effects of a hydroalcoholic extract (HE) of *Malva parviflora* (*M. parviflora*) in microglia. Primary microglial cells were isolated from wild-type CD1 mice and from 5XFAD, an AD mouse model. We demonstrated that the HE of *M. parviflora* possesses anti-inflammatory properties in neonatal mice microglia as it reversed the amoeboid phenotype (associated with activated microglia), inhibited the activation of NF-kB resulting from LPS exposure and decreased the expression of pro-inflamatory markers (CD86 and TNF-α) in the cortex of 5XFAD mice. Likewise, microglia cells treated with the HE of *M. parviflora* recovered the phagocytic capacity via a PPAR-γ/CD36 dependent mechanism that correlates with decreased load of β amyloid (βA) plaques in the cortex of 5XFAD mice and with improved learning and memory. These results suggest the therapeutic use of HE of *M. parviflora* to slow down the progression of AD by restoring microglial function.

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Keywords: Microglial cells, Alzheimer"s disease, *Malva parviflora*, PPARgamma, phagocytosis, neuroinflammation

Possible synaptic communication between neurons of Parafascicular and Central lateral intralaminar thalamic nuclei

Beatríz Sarahí Méndez Rodríguez, Dagoberto Tapia Ramírez, José Bargas Díaz y Elvira Galarraga Palacio*
División de Neurociencias, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, 04510, Ciudad de México. Tel.+52 55 5622-5621, egalarra@ifc.unam.mx.

The intralaminar thalamic nuclei, central lateral (CL) and parafascicular (Pf), are important sensory afferents to the striatum. In addition to their primary striatal projection, reciprocal synaptic connectivity between the neurons of Pf and CL nucleus has not been explored. The goal of this work was to find evidence that these intralaminar thalamic neruons are communicated.

We used mice of 4-6 week of age. Electrophysiological recordings used the patch clamp whole cell technique in both intralaminar thalamic nuclei neurons. Horizontal brain slices of 250 µm thickness including both intralaminar thalamic nuclei were kept in *in vitro* conditions with artificial cerebrospinal fluid bubbled with carbogen gas (95% O_2-5% CO_2). The recorded neurons were identified by intracellular labeling with biocytin. Synaptic responses were evoked with a bipolar concentric electrode of 50 µm in diameter in the nucleus opposite to the recording (CL-Pf). Field stimulation consisted of single square pulses of 0.1 ms of increasing intensity at 20 Hz. All experiments were done blocking the GABAergic inputs in the presence of gabazine (SR-95531, 10µM). We obtained synaptic responses in neurons of each intralaminar nuclei recorded after stimulation of the other nucleus. Synaptic responses showed two excitatory components: a glutamatergic component and an intrinsic component due to the activation of low threshold calcium currents (Ca_V3), that was blocked with NNC 55-0396 dihydrochloride (10 µM). Statistical analysis of maximum amplitude, half width duration and the area under synaptic responses were not significantly different when neurons recorded were from the Pf nucleus while stimulating the CL nucleus and viceversa.

The results are a first preliminary evidence of reciprocal synaptic connections between these nuclei. CONACyT: 251144 and DGAPA-UNAM: IN201517.

"Effect of D-β-hydroxybutyrate in autophagy induction by excitotoxicity"

Luis Angel Montes Ortega, Teresa Montiel Montes and María de Lourdes Massieu Trigo. *División de Neurociencias. Instituto de Fisiología Celular. Universidad Nacional Autónoma de México (UNAM). Ciudad Universitaria CP 04510. Ciudad de México, México. Tel. +52 55 56225761*
amontes@email.ifc.unam.mx

The neurotoxic toxic effect induced by excitatory neurotransmitters, mainly glutamate, is known as excitotoxicity. This mechanism frequently occurs in many neurological disorders, such as stroke[1], epilepsy and neurodegenerative diseases as Alzheimer, Parkinson$_2$, Huntington[3] and Amyotrophic Lateral Sclerosis[4]. It has been observed that in an *in vivo* models of excitotoxicity that D-β-hydroxybutyrate (D-BHB), a ketone body produced by fatty acids metabolism in the liver, prevents neuronal damage and reduces lipidic peroxidation levels in the rat striatum[5,6], which correlates with decreased production of reactive oxygen species (ROS)[7]. Recently, our group has suggested that D-BHB stimulates the autofagic flux induced by glucose deprivation in an *in vitro* model, by preventing ATP decline and autophagosome accumulation, stimulating neuronal survival. This suggests that D-BHB protects cells against excitotoxicity and that autophagy plays an important role in this effect[6]. Autophagy is a process by which eukaryotic cells recycle their cytoplasmic material through lysosomal degradation to maintain cellular homeostasis and it is also activated as an adaptive response to stress. In this study, we have analyzed the effect of D-BHB on autophagy in a model of excitotoxicity, induced by NMDA administration in the rat striatum. The levels of LC3-II were used as index of autophagosome formation while p62 is involved in autophagosome degradation; these two proteins were measured by western blot 24 h after NMDA injection in order to investigate whether autophagy is activated and autophagic flux disrupted under these circumstances. Animals were divided in three groups, control group which had no treatment, NMDA injected and NMDA plus 2 D-BHB (125 mg/Kg), one dose was intravenously injected immediately after NMDA administration and a second dose was intraperitoneally injected 3 hours later. Results indicate that D-BHB enhances autophagosome formation as we observed an increase in LC3-II levels but according to p62 levels, D-BHB does not stimulate autophagic degradation. Hence, according to these data it seems that in this excitotoxicity *in vivo* model, D-BHB promotes atophagosome formation but does not stimulate the authophagic flux at least at this time after the excitotoxic lesion.

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"Alpha-mangostin attenuates inflammation induced by systemic LPS administration in C57BL/6J mice and ameliorates memory deficits in a transgenic mouse model of Alzheimer´s disease"

Miryam Nava Catorce, Gonzalo Acero Galindo, Goar Gevorkian. Instituto de Investigaciones Biomédicas, UNAM E-mail: mncchiqui@hotmail.com Teléfono: 5534820226 Instituto de Investigaciones Biomédicas, UNAM AP 70228 Ciudad Universitaria CP 04510 México.

Neuroinflammation is an important feature in the pathogenesis and progression of several neurodegenerative diseases such as Alzheimer´s disease, Parkinson´s disease, multiple sclerosis and amyotrophic lateral sclerosis. Neuroinflammation is characterized by reactive astrocytes and microglia, besides the increase in levels of pro-inflammatory cytokines in the brain. For that reason, the use of anti-inflammatory compounds has been proposed as an alternative for the treatment of neurodegenerative diseases. Currently, numerous studies are carried out with natural and synthetic compounds aimed to prevent or decrease the neuroinflammation involved in these disorders. Recently, alpha-mangostin (α-MG), a natural polyphenolic compound derived from the pericarp of mangosteen fruit, has gained interest because of its multiple properties including anti-bacterial, anti-oxidative, anti-inflammatory and anticancer activity, as well as acetylcholinesterase and Aβ peptide aggregation inhibitor capacity. Based on previous information, in this study we evaluated the anti-inflammatory effect of α-MG on neuroinflammation induced by peripheral LPS administration in C57BL/6J mice and its capacity to ameliorate memory deficits in a triple-transgenic mouse model of Alzheimer´s disease (3xTg-AD).

First, we observed that α-MG treatment diminished diarrhea and conjunctivitis signs observed in mice after the LPS administration. Then, we found that α-MG attenuated the increase in serum and brain IL-6 protein levels in LPS-treated mice. In addition, we demonstrated that α-MG is capable to reduce the increase in brain COX-2 levels induced by LPS. Interestingly, we found that increase in brain COX-2 levels occurred in vascular endothelial cells.

On the other hand, we investigated the α-MG effect on glial activation induced by peripheral LPS administration. We have shown that α-MG significantly decreased TSPO expression, a glial activation marker, in both cortex and hippocampus from LPS-treated mice. Moreover, we found that TSPO expression was increased in vascular endothelial cells from LPS-treated mice but not in mice fed with -MG prior to LPS challenge.

After documenting the anti-inflammatory effect of α-MG in the brain, we tested the effect of α-MG on behavior in 12-month-old 3xTg-AD mice. We found that α-MG-treated mice performed better than vehicle-treated mice in a novel object recognition test. Probably, this effect is associated to the anti-inflammatory activity from α-MG, however, other mechanisms may be involved.

In summary, our results show that -MG can attenuate neuroinflammation induced by peripheral LPS administration in C57BL/6J mice by reducing brain IL-6, COX-2 and TSPO levels. In addition, we shown that α-MG attenuates vascular endothelial cell activation induced by LPS-treatment, which are important players in brain inflammation.

Finally α-MG is able to ameliorate memory impairment in a triple-transgenic mouse model of Alzheimer"s disease.

Effect of ethanol on the expression of *Creb* and *Xbp1* genes in hipocamppus of CD1 (ICR) mice and its relation with long-term memory

Jatziry Daniela Ocampo Ulloa, Anayeli Rojas Dircio, Eduardo Castañeda Saucedo, Martha Isela Barragán Bonilla and Carlos Ortuño Pineda.
Laboratory of Nucleic Acids and Proteins. Faculty of Biological Chemistry Sciences. Autonomous University of Guerrero.
Corresponding author: s/n Lázaro Cárdenas Avenue, 39087. Tel: 5531543508, Email: carlos2pineda@hotmail.com (C. Ortuño).

Abstract
Ethanol is the main component of alcoholic beverages, causing damage to the different regions of brain when consumed. The hippocampus is one of the most sensitive to the effect of ethanol, as a consequence memory is altered. A significant level memory regulation is the expression of genes encoding proteins such as cAMP Response Element-Binding protein (CREB) and X-box Binding Protein-1 (XBP1). The goal of this study was to demonstrate the effect of ethanol on the expression of genes involved in the long-term memory regulation (*Creb* and *Xbp1*), in the hippocampus of CD1 (ICR) mice, and to demonstrate their relation with long-term memory. Adult males CD1 (ICR) mice (n= 48) received acute treatment with 4.6 µM ethanol to evaluate their effect on the changes in the levels of expression of *Creb* and *Xbp1* genes by RT-PCR; and its effect on the long-term memory process by the novel object recognition task. RT-PCR experiments showed increased levels of expression of *Creb* and *Xbp1* in the hippocampus of mice treated with ethanol. On the other hand, the novel object recognition task showed that ethanol impairs long-term memory. The results suggest that acute treatment with ethanol modifies the expression levels of the *Creb* and *Xbp1*, and this is directly related to the regulation of long-term memory.

Key words: Ethanol, CREB, XBP1, memory, hippocampus.

Neuronal activity of primary visual cortex is altered in a genetic mouse model of autism (SHANK3)

Ortiz Cruz Carlos Alberto, Linares García Carlos Iván, Ramiro-Cortés Yazmín
Instituto de Fisiología Celular, UNAM., Circuito Exterior s/n, Del. Coyoacán, Ciudad Universitaria, 04510 Ciudad de México.
Tel. 56225697 email. yramiro@ifc.unam.mx

SHANK3 is a scaffolding protein localized in the postsynaptic density of glutamatergic synapses. Haploinsufficiency of SHANK3 by deletions and point mutations causes Phelan-McDermid Syndrome (PMS), an autism spectrum disorder (ASD) considered a synaptopathy. Humans with ASD show activity disturbances in cortical regions, like increased neural activity in visual processing brain areas, and atypical sensory processing such as discrepancies in visual perception, compared with no autistic people. However, underlying neural mechanisms remain unknown.

Herein, our first approach was determine how is the neuronal activity of primary visual cortex in a genetic mouse model of PMS, which present a deletion of Shank3 in one allele, just like in humans. We hypothesized that the haploinsufficiency of Shank3, which affect the synaptic function, may be altering the neural activity of V1 neurons.

In order to validate our hypothesis, we assessed the neural activity in layers 2/3 of V1 from heterozygous ($SHANK3^{+/-}$) and wild-type ($SHANK3^{+/+}$) awake head-fixed mice in response to a visual stimuli (gratings in eight orientations). We recorded the neural activity *in vivo* using genetically encoded calcium indicator (GCaMP6f) and two-photon microscopy through a cranial window in V1.

Our results shown by the first time that neural activity evoked by the visual stimulation is different in $SHANK3^{+/-}$ in comparison to wild-type. Specifically, we found a bigger proportion of responsive neurons in heterozygous mice in comparison to wild-type. Furthermore, we carried out analysis to determine the tuning responses of these neurons, finding that neurons from heterozygous mice are more selective for orientation and less selective for direction in sharp contrast to wild-type.

Based on the results outlined above, we want to further examine how the haploinsufficiency of SHANK3 may be impairing the brain function that is important for learning. In order to assess this, we propose to apply a visual discrimination task using a Go – No go paradigm for awake head-fixed mice. Our hypothesis is that $Shank3^{+/-}$ mice may have a low performance, related to the alterations in the neural activity.

TIME-DEPENDENT MITOCHONDRIAL TRANSLOCATION OF THE GLUCOCORTICOID RECEPTOR DURING THE CONSOLIDATION OF A PROCEDURAL MEMORY

Rogelio Pegueros Maldonado[1], Santiago Martin Pech Pool[2], Maricela Luna Muñoz[2], Mauricio Díaz Muñoz[2], and Gina Lorena Quirarte[1]
1Departamento de Neurobiología Conductual y Cognitiva, 2Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, UNAM Campus Juriquilla, Querétaro, Querétaro. México. C.P. 76230
Datos del autor principal
Dirección: Paseo de Libero #116, Res. Caletto, Juriquilla, Querétaro, México.
Tel. 4425989593
Correo electrónico: rogelio_pegueros@hotmail.com

Glucocorticoids facilitate memory consolidation (Medina et al., 2007; Quirarte et al., 2009) through the activation of glucocorticoid receptors (GRs), which act as transcription factors that induce *de novo* protein expression and activation of diverse signaling pathways (Kumar & Calhoun, 2008). The role of GR in neural plasticity and memory consolidation is well known; however, the whole spectrum of its functions is far from understood. It has been shown that GRs can translocate into the mitochondria in the brain (Moutsatsou et al., 2001), where they act as a transcription factor and enhance its activity (Du, McEwen, & Manji, 2009a); given the fact that mitochondrial activity is a primary factor for neural plasticity and memory, it is important to study the relationship between GR translocation and mitochondrial activity in a learning context. We studied this relationship, including the mitochondrial membrane potential in the dorsal striatum of a group of rats trained in the cued-water maze task, as well as in swim groups that were exposed to the water maze apparatus without the platform or the cue, and an intact group that stayed in its home box until the time of sacrifice. The results showed that both GR translocation into mitochondria and mitochondrial membrane potential showed a peak at 1.5 h after training. These findings suggest that the translocation of GR into mitochondria and the mitochondrial membrane potential have a functional convergence during memory consolidation. We thank the technical assistance of Norma Serafin, Martha Carranza, Olivia Vázquez, Martín García, Leonor Casanova, Lourdes Lara, and Ramón Martínez. Supported by PAPIIT-UNAM (IN204118) and CONACyT (251634).

Effect of viral maternal infection in structure, function and development of central nervous system.

Uriel Pineda Solís, Gustavo Pedraza-Alva, Leonor Pérez-Martínez .
Instituto de Biotecnología, Universidad Nacional Autónoma de México.
Av. Universidad 2001, Chamilpa, CP 62210, Cuernavaca, Morelos.
urielps@ibt.unam.mx, Correspondence: leonor@ibt.unam.mx

Psychiatric disorders, like schizophrenia and autism among others, have been shown to have multiple origins that range from genetic alterations to environmental influences like adverse experiences during childhood or insults during early development. However, a common denominator in this sufferings is the disturbance of different inflammatory mechanisms in the central nervous system (CNS). These alterations include chronic inflammation (even systemic), immune imbalance and, of course, disturbances in microglial activation.

Interestingly, it has been established that idiopathic cases of both disorders, which are the most common, could have their origin due an alteration in CNS development at early age. Neurodevelopment hypothesis, one of the most compelling proposals, point that systemic inflammation in the mother during pregnancy, caused by various factors like viral, bacterial or parasite infections; obesity and/or gestational diabetes; or another inflammatory condition, can signal or transmit towards the developing fetus, interrupting the proper formation of CNS or leaving some brand that lead to the development of schizophrenia or autism in the adult individual.

To induce a maternal infection, here we injected with Poly I:C (double strand RNA) on pregnant C57 female mice to activate mother"s immune response to a viral infection. As it has been reported, this treatment triggers a systemic inflammation during second third of pregnancy.

Once pregnancy came to the end and offspring came to adult age, we performed behavioral and social tests to determinate behavioral alterations associated with the maternal inflammatory insult. Likewise, the serum levels of inflammatory molecules were evaluated to determine if there was still traces of mother"s inflammatory response on offspring.

Current experiments are aimed to determinate neuronal density and the existence of possible alterations on cerebral structure and neurochemical signaling: specifically neurotransmitters whose signaling pathway is altered in diseases like schizophrenia and autism.

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Expression of the GluN1, GluN2B and GluN3A subunits of the NMDA receptor and zinc transporter-1 in the vestibular system of chicken.

Ana María Ramírez Ramírez, Eduardo Monjaraz Guzmán, Jorge Cebada Ruiz and Amira Flores Urbina. Instituto de Fisiología de la Benemérita Universidad Autónoma de Puebla. 14 Sur 6301, Jardines de San Manuel CP: 72592 Puebla, Pue. Tel: 2295500 Ext: 7303 Email: amrayo@yahoo.com.mx VIEP-BUAP-CA-119 (2018)

Abstract.
During embryonic development and in the first weeks of postnatal development of mammals, reptiles and birds in different areas of the CNS, the presence and function of NMDA receptors (NMDAR) has been detected. Recently, evidence about a direct interaction between zinc transporter-1 (ZnT-1) and a subunit (GluN2A) of the NMDAR has been discovered. The purpose of this study was to observe the presence of GluN1, GluN2B and GluN3A subunits and ZnT-1 during the development of the chicken"s vestibular organ (*Gallus domesticus*). We performed RT-PCR assays in order to assess levels of mRNA expression encoding the different subunits of NMDA receptors in both the crista ampullaris and the vestibular ganglion. This study allowed us to establish the existence of a pattern of expression of the different subunits, depending on the age of embryonic (E15, E18, E21) and posthatching (P10) chicks. For each experimental series 72 crests and 24 ganglions of chicks were used, and total RNA from the crista ampullaris and vestibular ganglion was isolated using Quick-RNA^{TM} MiniPrep (Zymo Research, USA), according to the manufacturer"s directions. The concentration of total RNA for each sample was determined with absorbance measurements at 260 and 280 nm (Biophotometer, Eppendorf, USA.). cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA). Obtained cDNA was subjected to the PCR protocol to verify its identity, amplification products were resolved by electrophoresis in 1.5% agarose gels and visualized with ethidium bromide staining, and the intensities were then measured by scanning the gel using the ChemiDoc Gel Documentation System (Bio-Rad, USA). RT-PCR experiments revealed the presence of the GluN1, GluN2B and GluN3A subunits and also the ZnT-1. These subunits and the transporter increased their presence at near hatching (E21) and post-hatching ages (P10). Our results suggest that NMDAR could modulate the spontaneous activity of the vestibular afferent fibers in an age-dependent way in the chicken"s inner ear.

A *Malva parviflora´s* fraction ameliorates the spatial learning and memory impairments resulting from neuroinflammation

Cristina E. Ramírez Serrano[1], Maribel L. Herrera-Ruiz[2], Jesús E. Jiménez Ferrer[2], Alejandro Zamilpa Alvarez[2], Gabriela Vargas-Villa[2], Jaime Tortoriello García[2], Gustavo Pedraza-Alva[1] and Leonor Pérez-Martínez[1]
[1]Laboratorio de Neuroinmunobiología, Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología, Universidad Nacional Autónoma de México (UNAM), Cuernavaca, Morelos 62210, México. [2]Centro de Investigación Biomédica del Sur, Instituto Mexicano del Seguro Social (IMSS), Xochitepec, Morelos CP 62790, México. Correspondence: leonor@ibt.unam.mx

Alzheimer´s disease (AD), the most common kind of dementia, is a neurodegenerative disorder characterized by cognitive impairment and behavior disturbances. The etiology of this condition is not well characterized; however, it is known that the inflammatory processes developed before and during its progression play a negative role on memory and learning. Furthermore, it is considered that certain conditions involving a chronic pro-inflammatory state at the periphery level as diabetes and hypertension, are important risk factors to develop this disease. Based on this, new strategies to treat AD have been proposed. Among them, the use of non-steroidal anti-inflammatory drugs (NSAIDs) have shown to decrease the incidence of this disease when taken during long periods; unfortunately, its prolonged use results in adverse secondary effects. In this context, the secondary metabolites of plants with anti-inflammatory properties have become of great interest. Particularly, it has been demonstrated that a hydro-alcoholic extract of *Malva parviflora* (MpHA) has anti-inflammatory effect and is capable of improving the cognitive deficit present in an AD mouse model. To further characterize the *Malva parviflora* compounds, we generated a fraction from dichloromethane extract, which constitutes a less complex mix of compounds than the MpHA but maintains its biological activity. This approach also allowed us to isolate a single compound with anti-inflammatory effect. Importantly, the MpF10 and the isolated compound ameliorate the spatial learning and memory impairments, at the same time that reduces the reactive astrogliosis in a murine model of LPS mediated neuroinflammation. Therefore, we propose that the compounds present in MpF10 represent an alternative to treat neuroinflammation and AD.

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Keywords: Alzheimer"s disease, *Malva parviflora*, neuroinflammation.

Gender comparation of the anxiolytic like effect of the administration the infusion of Justicia spicigera leaves in Wistar rats

Ana Raquel Ramos-Molina [1,2], Rosa Isela García-Ríos [2], Armando Mora-Pérez[2], Cesar Soria-Fregozo [2], Daniel González Torres[2] y Andrea Martínez González[2]

[1]Estudiante de Posgrado en Ciencia y Tecnología. Centro Universitario de los Lagos de la Universidad de Guadalajara. an.raquel1806@gmail.com.
[2]Laboratorio de Ciencias Biomédicas/Área Psicobiología e Histología. Centro Universitario de los Lagos de la Universidad de Guadalajara. Av. Enrique Díaz de León N° 1144, Col. Paseos de la Montaña, C.P. 47460, Lagos de Moreno, Jalisco, México. Teléfono: +52 (474) 742 4314, 742 3678 Fax Ext. 66527. rosai_garios@yahoo.com; csoria@culagos.udg.mx ; mora_uv@yahoo.com.mx.

Anxiety is an emotion considered as a normal response of adaptation and survival of the organism to some imminent danger. When this response exceeds the limit or occurs with no reason, affecting the life quality of the subject is considered a pathological anxiety. Due to the collateral problems presented by anxiolytic treatments and the difference in response by gender, many alternative treatments have been sought, like the traditional medicine use. Such as the case of Justicia Spicigera or muicle, a plant used in general as a "nervous tonic". It has been identified that 12 mg/kg of the infusion of Justicia spicigera leaves (IHJs) produces an anxiolytic like effect in female cycling rats similar to diazepam. However, it is unknow if this effect also occurs in male rats, a possibility that needs to be explored, it has been reported that females respond better than males to anxiolytic treatments.

Objective: to identify if there is a difference of gender in the anxiolytic effect that produces the administration of IHJs and compare it with diazepam in Wistar rats, subjected to the elevated plus maze test and locomotor activity.

Method: 30 male and 30 female rats into phases metestrus/diestrus (M-D) were used. These were divided into 3 experimental groups by gender (vehicle, purified water 1.8 ml / kg v.o (VEH), 12 mg / kg IHJs v.o.; 2 mg / kg diazepam, i.p (DZP) as positive control).

Results: In general, male rats remain more time in the open arms than female rats in the (M-D) ($p=0.015$) phases. The administration of 12 mg/kg de la IHJs significantly increased the remain time in the open arms, both groups males and females in the phases of M-D ($p<0.001$) compared to the VEH and similarly to DZP both of their respective gender. On the other hand, it was identified that 12mg/kg of the IHJs increased the risk time assessment ($p=0.001$) in males and in females, in which, in a similar way to the DZP. Without finding significant statistical differences ($p=0.545$) in the number of crossed squares.

Conclusion: The anxiolytic like effect of the IHJs is not gender dependent. However, it has a greater pharmacological efficacy in males than in females with low concentrations of ovarian hormones, probably exerting its effect through GABAergic and / or serotonergic mechanisms.

Ivermectine and Ethanol Effect Evaluation on the Human P2X4 Receptor

M. C. Naybi Nikte-ha Requejo Mendoza, D. C. Carlos Barajas López, Lic. Rosa Espinosa Luna, D. C. María Guadalupe Nieto Pescador
Instituto Potosino De Investigación Científica y Tecnológica (IPICYT) - Facultad de Ciencias Químicas, Universidad Juárez del Estado de Durango, (FCQ, UJED)
Camino a la Presa San José 2055.Col. Lomas 4 sección CP. 78216. San Luis Potosí S.L.P.
Tel: (618) 182 95 37
naybi.requejo@ipicyt.edu.mx

Alcohol intake can induce diverse negative health effects, affecting organs and tissues (OPS, 2015b).

Pharmacological and preclinical studies using murine models have demonstrated that IVM has a diminishing effect on alcohol consumption, suggesting it as a potential therapeutic drug for alcohol use disorders treatment. IVM is an approved drug by the FDA for the treatment of parasitic diseases; however, outside its antiparasitic effect, IVM is a positive and highly selective modulator of the P2X4 purinergic receptor, besides it counteracts the inhibitory effects that ethanol has on the said receptor. Within the family of P2X receptors, P2X4R is the most widely expressed in the Central Nervous System (CNS) and has the highest sensitivity to ethanol (EtOH), in addition, it is present in the neurons that make up the aversion system and reward, so it is believed that plays an important role in the regulation of this system and therefore in the generation of alcohol dependence.

However, at the moment there is no reported work of the pharmacological characterization of IVM and EtOH effects on human P2X4 receptor; while there is evidence of the different pharmacological responses to the same drug, between orthologues of P2X4R.

The objective of this work was to evaluate the pharmacological effects of EtOH and IVM on human P2X4R by means of the Electrophysiological technique of Fixation of Voltage with two electrodes (TEVC); using as a system of heterologous expression, the frog oocytes of the species Xenopus laevis. Our results suggest that the IVM presents a neutralizing action of the effects of EtOH on the P2X4R channel of human, likewise it is noteworthy that the results obtained during the realization of this thesis work, differ from the reported until now, being the EC50 for ATP and IVM greater and IC50 for EtOH lower than those reported on rat P2X4R, so that the taste for alcohol in humans could be diminished.

Cortical Persistent Activity In Pyramidal And Interneurons Of Layer 5 Motor Cortex "*In vitro*"

Rosa María Reyes Chapero, Dagoberto Tapia Ramírez,
José Bargas Díaz y Elvira Galarraga Palacio*.
Neurociencia Cognitiva. Instituto de Fisiología Celular. Universidad Nacional Autónoma de México.
Ciudad de México, 04510. Tel: 55 5622-5621, egalarra@ifc.unam.mx

Persistent activity is a fundamental form of circuit dynamics, which is important for many behavioral functions. This activity is generated intrinsically in the cortex and can be preserved in cortical preparations "*in vitro".* The cerebral cortex is characterized by a densely interconnected network of glutamatergic pyramidal neurons and GABAergic interneurons. Proportional balance between excitation and inhibition varies during cortical persistent activity and its loss leads to the development of diverse neuropathologies. The importance of fast GABAergic inhibition in controlling persistent activity is supported by the fact that blocking $GABA_A$ receptors transforms control UP-states into epileptiform bursts. There are differential roles for interneurons and pyramidal cells in the generation of control persistent activity. In this study, we recorded the firing pattern of morphologically identified pyramidal cells and interneurons of layer 5 motor cortex during cortical persistent activity. Whole cell-patch clamp recordings were performed with borosilicate glass pipettes filled with internal solution with the following (in mM): (in mM): 120 KMeSO4, 2 MgCl2, 10 HEPES, 10 EGTA, 1 CaCl2, 0.2 Na2ATP, 0.2 Na3GTP, 1% biocytin, and 290 mOsmol/L. Data acquisition used software Im-Pach and digitized data were imported for analysis and graphing into Origin 7.

Neurons were identified according to their morphological and electrophysiological phenotypes as well as immunocytochemistry. Molecular markers (red or green fluorescent proteins) helped to identify parvalbumin positive interneurons (PV+) in transgenic animals. Cortical slices generated persistent activity with external solution (ACSF) containing a lower concentration of magnesium and $GABA_A$ receptor antagonist bicuculine [10 μM], in this condition, both types of neurons exhibited paroxysmal depolarization shifts (PDS). In PV+-interneurons the duration of this PDS is longer (1-6 seconds), the firing frequency is higher (161Hz) and exhibit a depolarization-block phase, with a burst frequency of 22 Hz. Pyramidal cells exhibit a shorter PDS (1-3 seconds) with lower firing frequency (111Hz), and burst frequency of 32 Hz. These results show the differences in the PDS of interneurons and pyramidal cells in motor cortex. These differences distinguish the type of neuron activated in order to study their specific roles in the regulation of persistent cortical activity.

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PROLACTIN MODIFIES BLOOD-BRAIN BARRIER PERMEABILITY *IN VITRO*

Josue Rivera[1], Rodrigo Aroña[1], Miriam Ulloa[1], Fernando Macías[1], Carmen Clapp[1], Edith Arnold[1,2], Gonzalo Martínez de la Escalera[1*].
[1]Department of Cellular and Molecular Neurobiology; [2]CONACYT-Catedrática, Neurobiology Institute, Universidad Nacional Autónoma de México, Campus UNAM Juriquilla, Querétaro, México. (55) 5623 4029, gmel@unam.mx

The blood-brain barrier (BBB) is form by tight junctions between the endothelial cells. The BBB permeability is regulated by pericytes, astrocytes, and microglia cells, through molecular factors that modulate the expression and maintenance of tight junction proteins. It has been described that the hormone prolactin (PRL) exerts regulatory effects on endothelium proliferation and apoptosis, as well as in the permeability of the mammary endothelium. However, the putative role that PRL plays in the permeability of the BBB have not been well characterized. The purpose of this project is to investigate the participation of PRL on the regulation of the function of the BBB. We investigated the effect of PRL (0.1, 1, 10, 50 and 100 nM) on the permeability of endothelial cells in an in vitro model of the BBB, by measuring transendothelial electrical resistance (TEER) at 5, 10, 20, 40 and 1440 min (24 h) under three different conditions on Transwell inserts: 1) primary mono-cultures of cerebral microvascular endothelial cells (EC) seeded in the inner part of the insert, 2) co-cultures of EC with astrocytes (AS) seeded in the lower part of the membrane of the insert, and 3) co-cultures of EC and AS placed on well plates containing neurons. TEER values for the mono-cultured EC inserts on basal conditions were significantly lower in comparison with the co-culture models. PRL treatments significantly increased the transendothelial resistance in a concentration-dependent manner in the three conditions tested. Interestingly, the maximum effect was observed between 5 and 10 min, and values returned to control levels by 20 min post stimulation. These results show that PRL decreases endothelial permeability in a concentration-dependent manner.
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EFFECTS OF A NEONATAL STRESS - IMMUNE CHALLENGE ON THE NEUROINMUNE SYSTEM OF THE HIPPOCAMPUS AND THE BEHAVIOR OF ADULT MALE RATS.

Luis Miguel Saavedra Pimentel[1,2], Alejandra Ochoa Zarzosa[1], Luz Torner[2].
Universidad Michoacana de San Nicolás de Hidalgo, (CMEB) [1]
Centro de investigación Biomédica de Michoacán (CIBIMI, IMSS)[2]
Camino a la arboleda #300 Colonia Exhacienda de San José de la Huerta. Morelia Michoacán.
Lmsp_603@hotmail.com

Introduction: Early life stress permanently affects the development of the central nervous system, and increases the vulnerability to develop psychological disorders such as anxiety and depression. These diseases are accompanied by inflammatory processes at the cerebral level, mediated mainly by glial cells. Also, severe infections at early ages seem to cause might psychological disorders and alter cognitive processes in adulthood.

Objective: To analyze the effects of early exposure to a double stress-immune challenge on the emotional behavior, learning, and on the neuroimmune system in an animal model.

Methodology: Four groups of male Sprague Dawley rats (n = 8) were used as follows: 1) control group + vehicle, 2) control group + Lipopolysaccharide (LPS), 3) maternal separation group (MS, 3 hours/day from postnatal day PN 1 to 14) + vehicle, 4) group MS + LPS. LPS was administered as an immune stimulus (0.5mg / kg) on PN14. The emotional state of the animals was analyzed starting at PN120 subjecting them to the elevated plus maze test, open field and forced swimming. Their spatial and non-spatial memory was evaluated using the object recognition and object placement tests. Immunostaining of brain sections from the 4 groups with specific antibodies (GFAP) were performed to analyze astrocyte density.

Results: The MS-vehicle and MS-LPS groups showed increased depressive - like behavior. The Control-LPS and MS - LPS showed an anxious - like behavior as adults. No effects on spatial and non-spatial learning were observed with MS and/or LPS challenges. In the hippocampal hilus, LPS causes an increase in the total cellular density of astrocytes, but no differences were observed in MS animals.

Conclusions: LPS causes alterations in glial cells related to neuroinflammation. Exposure to LPS and early MS lead to emotional disturbances (depression and anxiety), but does not affect memory in adulthood.

Tibolone improves memory and reduces beta-amyloid and Tau protein levels in the hippocampus of the triple transgenic mouse for Alzheimer's disease

Julia Segura-Uribe[1], Paola García de la Torre[1], Marvin Soriano-Ursúa[2], Juan Manuel Gallardo[3], Nadia Tzayaka Castillo-Mendieta[4], Christian Guerra-Araiza[4]
[1]Unidad de Investigación Médica en Enfermedades Neurológicas, [2]Escuela Superior de Medicina, Instituto Politécnico Nacional, [3]Unidad de Investigación Médica en Enfermedades Nefrológicas, [4]Unidad de Investigación Médica en Farmacología, CMN S XXI, IMSS;
Ciudad de México, México.
Av. Cuauhtémoc 330, Col. Doctores, Del. Cuauhtémoc. C.P. 06720. Ciudad de México. Tel. 56276900 ext. 21367. *E-mail*: christianguerra2001@gmail.com

Tibolone (TIB), a synthetic steroid, has been used for neuroprotection in several animal models. To assess the effect of tibolone in a triple transgenic model for Alzheimer's disease (3xTgAD), we evaluated the short-term memory as well as the content of the beta-amyloid peptide (βA 40) and Tau protein in the hippocampus and cerebellum of ovariectomized 3xTgAD females.

Three-month-old females from both wild type (WT) and 3xTgAD strains were ovariectomized. Ten days after surgery, four treatment groups were formed (n = 10): WT + vehicle (veh), WT + TIB (1 mg/kg/weight), 3xTgAD + veh and 3xTgAD + TIB (1 mg/kg/weight). Treatment was administered for three months. Once the treatment period ended, memory tests (recognition of objects in context (ROC) test) were performed. Subsequently, animals were sacrificed by decapitation. The hippocampus and cerebellum were dissected. Tissues were homogenized with a TBS buffer for the quantification of βA 40 and total Tau. Commercial ELISA kits were used. The analysis was conducted in accordance with the manufacturer's instructions.

TIB treatment improved memory in the 3xTgAD + TIB when compared to groups treated with veh. Furthermore, it was observed that TIB treatment decreased the content of 40 βA and total Tau in the hippocampus of 3xTgAD mice compared with veh-treated groups. Conversely, there were no significant changes in the content of both proteins in the cerebellum or WT strain groups.

These results suggest that chronic treatment with tibolone presents neuroprotective effects and delays Alzheimer's disease neuropathology

Antidepressant-like effect of the chronic administration of the infusion of Justicia spicigera leaves on male rats: A comparison with imipramine and fluoxetine.

Cesar Soria-Fregozo [1], Rosa Isela García-Ríos [1], Carlos Andrés Contreras Salazar [1] y Armando Mora-Pérez[1].
[1]Laboratorio de Ciencias Biomédicas/Área Psicobiología e Histología. Centro Universitario de los Lagos de la Universidad de Guadalajara. Av. Enrique Díaz de León N° 1144, Col. Paseos de la Montaña, C.P. 47460, Lagos de Moreno, Jalisco, México. Teléfono: +52 (474) 742 4314, 742 3678 Fax Ext. 66527. csoria@culagos.udg.mx

Introduction: The World Health Organization (2013) estimated that by the year 2020, depression will be the second cause of disability worldwide. It affects two women for every man; however there is a higher rate of suicide in men. Due to the long therapeutic latency and the adverse effects of antidepressant treatments, the use of traditional medicine has increased. In this sense, the infusion of the leaves of Justicia spicigera (ILJs) or "Muicle" is used to treat "tristeza" (dysthymia, the mildest form of depression), among other things. It has been identified that chronic administration of 24 and 48 mg/kg of ILJs produce an antidepressant-like effect in the forced swimming test in female rats during the metestrus-diestrus (M-D) phases of the estrous cycle, phases characterized by low plasma levels of ovarian hormones, a condition that seems to be determinant for the effect of the ILJs to be present, since it was not detected with the acute administration of the ILJs during the proestrus-estrus (P-E) phases of the estrous cycle, characterized by high concentrations of ovarian hormones. However, it is unknown whether this effect also occurs in male rats, because they have a low concentration of hormones such as progesterone and estradiol.

Objective: Evaluate the effect of the administration for 21 days of 6 mg/kg and 48 mg/kg of the ILJs, and compare it with fluoxetine (10 mg/kg) and Imipramine (15 mg/kg) in male rats evaluated on the locomotor activity and forced swimming test. Methods: Five experimental groups (n=8/group) of adult male Wistar rats were used, which received: a) vehicle group (purified water, 1.8 ml/kg), b) group 6 mg/kg of the ILJs, c) group 48 mg / kg of the ILJs, d) fluoxetine group 10 mg/kg (FLX) and e) imipramine group 15 mg /kg (IMI) during 21 days of oral treatment. Results: No significant differences were found in the latency at the first immobility (p = 0.281). However, it was found that 6 and 48 mg /kg of the ILJs significantly reduced immobility (p <0.001) in a similar way to IMI and FLX and in comparison with the VEH. Without modifying the locomotor activity (p = 0.097).

Conclusion: The 21 day administration of 6 and 48 mg /kg of the ILJs produces an antidepressant-like effect in the male rat Wistar strain, without modifying the locomotor activity and in a similar way to clinically effective antidepressants. This suggests that the metabolites contained in the plant exert their effects in a manner not dependent on gender and in the case of males with doses lower than the minimum effective detected in females, probably due to an interaction with testosterone.

Analysis and identification of allosteric ATP-binding sites in human P2X1 receptors

Estefania Tejeda-Jaramillo, Sergio Nemorio Hidalgo-Figueroa, Rosa Espinosa-Luna, Carlos Barajas-López.

P2X receptors are no-selective cationic channels gated by adenosine triphosphate (ATP); they are involved in many physiologic processes like neurotransmission, nociception and inflammation (Burnstock, 2006; Chataigneau et al., 2013). The pharmacodynamics of these receptors is variable among each of the subtypes, regarding the P2X1 receptor pharmacodynamics, it has been described by electrophysiological studies as a unique response sensitive to low ATP concentrations (nM), response that rapidly desensitize. A recent study shows that these receptors generate a quite different current when high ATP concentrations (mM) are used (https://doi.org/10.1016/j.ejphar.2016.10.033). This current increased slowly its amplitude during the agonist exposure. This latter current, but no the desensitizing response, is preserved in the splicing variant of hP2X1 (hP2X1*del*), which supports the hypothesis that the deleted segment in hP2X1*del* is related to the high sensitive ATP binding site but maintains the low sensitive binding site. To study the P2X1 and P2X1*del* 3-D structure it would allow us to explore ATP recognition sites and understand the molecular basis of the modulation and opening of their channels. However, its structure is unknown. We used by first time homology modeling technique based on reference structures of the P2X family (hP2X3 and hP2X7) to determine the 3-D structure of the hP2X1 and hP2X1*del* receptors, the assembly of the receptors was performed using the MolSoft 3.8-5 and Pymol v1.8.6.0 programs together. To validate the models, an amino acid energy analysis was performed as a method of local validation and Z-score analysis and Ramachandran graphs as general validation methods for each receptor with the ProSa server (Protein structure analysis) and MolProbity (Davis et al., 2007). To explore the ATP binding sites in the hP2X1 receptor, a cavity analysis was performed with the PyMol v1.8.6.0 program. Finally, molecular docking studies were carried out on the hP2X1 and hP2X1*del* receptors in the proposed binding sites with the Autodock 1.5.6rc3 program. Our results suggest the existence of an additional ATP binding site in the hP2X1 receptor that would be conserved in the hP2X1*del* receptor.

A post-mortem proteomic analysis of the prefrontal cortex of individuals with completed suicide and positive toxicology to alcohol.

A post-mortem proteomic analysis of the prefrontal cortex of individuals with completed suicide and positive toxicology to alcohol.

Jonatan Alexis Torres-Campuzano, Nora Andrea Gutiérrez-Nájera, Manuel Alejandro Rojo-Romero, Ana Luisa Romero-Pimentel, Mirna Edith Morales-Mendoza, Fernando García-Dolores, Roberto Cuauhtémoc Mendoza-Morales, Alejandra Mendoza-Larios, Ernesto Díaz-Otáñez, Elí Elier Gonzáles-Sáenz, José Humberto Nicolini-Sánchez.
Instituto Nacional de Medicina Genómica
Av Universidad 3000, Cd. Universitaria, Coyoacán, 04510 Ciudad de México, CDMX
Phone: 5526897770
E-mail: johnny-tc@ciencias.unam.mx

Abstract: Suicide has become a public health problem worldwide, therefore a lot of research has been done in order to understand the biolological factors and processes that contribute to the development of this phenomenon. Suicide behaviour has a multifactorial origin and it could be influenced by different factors such as alcohol abuse and molecular changes in the brain. Almost the 50% of people that committed suicide was positive to alcohol intoxication. Molecular studies such as in proteomics can be useful to identify alterations in proteins and candidate genes related with this behaviour. However protemic studies on the human brain are scarce. The aim of this study was to determine differences of the protein profile of the prefrontal cortex of suicide victims, suicide victims with positive toxicology to alcohol, and controls (people that died of another cause). We performed a proteomic analysis of the prefrontal cortex of 13 subjects. We obtained the protein profile by a 2D-PAGE. To determine the diferences we compared the three conditions to each other with the 2D gels images obtained with Sypro Ruby and we performed the comparsion with the PDQuest software. Finally we identified the proteins by mass spectrometry. We found proteins that could be involved in neurobiological proceses of suicide behaviour and alcohol abuse, such as mitocondrial aldehyde dehydrogenase 2 and glial fibrillary protein. This study could provide new information about the comorbidity of alcohol intoxication and suicide.

Transcriptional and Chromatin Accessibility of Dopaminergic Differentiation from Induced Pluripotent Stem Cells

Jessica G Herrera-Gamboa[1], Raquel Cuevas Díaz Durán[1], Tonatiuh Barrios Garcia[1], Francisco Islas Cisneros, Iván Velasco[2], Víctor Treviño[1,*]
[1]Tecnológico de Monterrey-Escuela de Medicina, [2]UNAM-Instituto de Fisiología, Monterrey, Nuevo León, México, Av. Morones Prieto
*vtrevino@itesm.mx

Mathematical modeling of temporal stem cell differentiation processes may help to understand important regulatory mechanisms. We are proposing models to predict the transcriptional state of cells given some stimuli. Nevertheless, the accuracy of our models is relatively low, so integrative analyses of diverse genomic data is necessary. In this work, we generated temporal transcriptional (RNA-Seq) and epigenetic data providing chromatin accessibility (ATAQ-Seq) from the stimulated differentiation of induced pluripotent stem cells to dopaminergic cells at 0, 14, and 28 days. We made an initial low-throughput run in a MiSeq system and a High-throughput run in a HiSeq system. The reads were processed in Galaxy and well stablished bioinformatics tools for data processing analysis. Our results confirm that we successfully generated RNA-Seq and ATAC-Seq libraries representing the process of differentiation. Our results also validate that dopaminergic cells were successfully obtained when compared to well-known gene markers. Interestingly, our RNA-Seq seems less noisy and more precise than published data. A differential analysis shows that, besides markers, we observed genes that turn on slowly and rapidly, that turn off slowly and rapidly, and many that are transitory mainly turning on and off and few turning off and on. We were able to extract useful information from ATAC-Seq data, which seems to match differentially expressed genes. Our data will be valuable for mathematical modeling of the differentiation process.

Analysis of differential potencies of secretagogues upon Growth Hormone (GH) regulation in vertebrates

Valeria Alejandra Urban Sosa, José Ávila Mendoza, Martha Elizabeth Carranza Salas, Maricela Luna Muñoz, Carlos Guillermo Martínez Moreno and Carlos Arámburo de la Hoz

Department of Cellular and Molecular Neurobiology, Institute of Neurobiology, UNAM, Campus Juriquilla, Querétaro, CP 76230. Tel (442) 2-38-10-65
urbanv@comunidad.unam.mx

The regulation of pituitary GH synthesis and secretion in vertebrates involve the participation of several peptide secretagogues (i.e. GHRH, TRH, PACAP, Ghrelin, GnRH, SST and IGF1) that interact in a complex manner. Apparently, their level of participation and potency upon GH mRNA expression and GH release vary along vertebrate evolution, as shown in this work, where we compared their effects and potencies in mammalian (rat), avian (chicken) and reptilian (iguana) pituitary cultures. Our results indicated that GHRH stimulated the secretion of GH with greater potency in iguana (405%) vs chicken (28.2%) or rat (28.9%); likewise, the expression of GH mRNA was larger in iguana (59.3 times), than in rat (15.1 times) or chicken (decreased 0.1 times). TRH had greater potency to stimulate GH secretion in chicken (14.2%), than in rats (decreased 21.2%) or in iguana (decreased 11.7%); whereas, in terms of GH mRNA expression, TRH was much more potent in iguana (179.2 times) than in rat (5.9 times) or chicken (3.5 times) pituitary cultures. PACAP showed a bigger effect upon GH release in iguana (107.7%) than in rat (9.5%) or chicken (decreased 3.4%); while it promoted GH mRNA expression more potently in iguana (28.1 times) than in chicken (5 times) or rat (1.7 times) cultures. Interestingly, while Ghrelin increased GH secretion more potently in iguana (61% increase) than in chicken (20.5%) or rat (decreased 17.4%), it inhibited the expression of GH mRNA in the three models studied (iguana decreased 0.03 times; chicken, 0.035 times, and rat, 0.58 times). GnRH was more potent stimulating GH secretion from iguana pituitaries (121%), than in chicken (17.4%) or rat (decreased 14.1%); whereas the expression of GH mRNA increased with greater potency in chicken pituitary cultures (2 times) than in rat (decreased 0.37 times) or iguana (decreased 0.74 times). On the other hand, Somatostatin inhibited GH secretion more potently in iguana cultures (decreased 92.1%), than in rat (decreased 20%) or chicken (decreased 14.8%), in a manner similar to the expression of GH mRNA: iguana (0.005 times), rat (0.11 times), chicken (0.78 times). Finally, IGF-I decreased GH secretion with greater potency in iguana pituitary cultures (83.7%) than in rat (19.9%) or chicken (0.37%); whereas. it decreased the expression of GH mRNA with greater potency in the chicken pituitary cultures (0.32 times) than in iguana (0.6 times). These results suggest that the involvement of several peptide secretagogues upon GH regulation vary during vertebrate evolution. (Supported by PAPIIT-IN201817)

Atomoxetine in neuron-like cells produces oxidative stress and alters mitochondrial function

Daniela Vázquez González, Denise Gómez Bautista, Sonia Carreón Trujillo, Raquel González Pérez and Juan Carlos Corona*

Laboratory of Neurosciences, Hospital Infantil de México Federico Gómez. Dr. Márquez 162, Colonia Doctores, Delegación Cuauhtémoc, C.P. 06720, Mexico City, Mexico. Tel: 52289917 ext. 2352. *E-mail: jccc93@gmail.com

Attention-deficit/hyperactivity disorder (ADHD) is the most common neurodevelopmental disorder in childhood and is characterized by inattention, impulsivity and hyperactivity. ADHD is the most frequently diagnosed condition in children, affecting more than 5 million Mexicans. Atomoxetine (ATX) is a non-psychostimulant drug used in the treatment of ADHD, is a selective norepinephrine reuptake inhibitor. However, it has been shown that ATX has additional effects beyond the norepinephrine reuptake inhibition, affecting several signal transduction pathways and alters gene expression, for example: it was demonstrated that ATX acts as an NMDA receptor blocker; inhibits G-protein-activated inwardly rectifying K^+ channels; up-regulates BDNF expression in the prefrontal cortex, thus influencing synaptic plasticity and cognitive function; increases the expression of GABA A receptor subunit as well as ubiquinol–cytochrome c reductase complex (complex III) core protein 2 and synaptosomal-associated protein of 25 kDa, which is an ADHD candidate gene and an important vesicle protein involved in axonal growth, synaptic plasticity and regulation of neurotransmitter release (Prog Neuro-Psychopharmacol Biol Psych 40:221, 2013; Brit J Pharmacol 160:283, 2010; Neuropsychopharmacol 35:1560, 2010; Pharmacol Res 62:523, 2010). Therefore, we have studied whether ATX has an impact on oxidative stress and mitochondrial function in human differentiated SH-SY5Y cells exposed over a range of concentrations. Our data showed that concentrations between 20 and 50 μM of ATX, produced free radical generation and alterations on mitochondrial mass, membrane potential and increase autophagy. Thus, depending of the ATX concentration used, there are alterations on oxidative stress and mitochondrial function, indicating that ATX produces additional effects beyond the norepinephrine reuptake inhibition.

Effect of M4 on Oxidative Stress in intra hippocampal injected animal model of Alzheimer´s disease

Authors: Daniel Miguel Angel Villalobos Acosta[1], Maricarmen Hernández Rodríguez[1], Brenda Chimal Vega[1], Enrique Querejeta Villagomez[2] and Martha Cecilia Rosales Hernández[1].
[1L]aboratorio de Biofísica y Biocatálisis, Sección de Estudios de Posgrado e Investigación, Escuela Superior de Medicina, Instituto Politécnico Nacional, Plan de San Luis y Díaz Mirón s/n, Casco de Santo Tomás, Distrito Federal 1340, México.
[2] Laboratorio de Neurofisiología, Sección de Estudios de Posgrado e Investigación, Escuela Superior de Medicina, Instituto Politécnico Nacional, Plan de San Luis y Díaz Mirón s/n, Casco de Santo Tomás, Distrito Federal 11340, México.
Correspondence: marcrh2002@yahoo.com.mx; Tel.: +55 57296000 ext. 62809

Objective: To assess the M4 compound, on the oxidative stress and betaamyloid 1-42 ($Aß_{1-42}$) concentration in an Alzheimer animal model.

Methods: Wistar Rats were injected into dorsal hippocampus and were divided in four groups (*n*=6), as follow: Control Group(CG) with synthetic cerebrospinal fluid, ß-amyloid group (GßA) with fibril $Aβ_{1-42}$ without treatment, ß-amyloid group+galantamine(GGA) with fibril Aβ1-42 and treated with galantamine (I.P. 2 mg/Kg/day) and ß-amyloid group + M4(GM4) with fibril $Aβ_{1-42}$ and treated with M4 (I.P. equimolar relation with galantamine). After 35 days the animals were sacrificed and oxidative stress parameters (GSH, LPO and FR) and $ßA_{1-42}$ concentration were evaluated in hippocampus samples.

Results: Our results demonstrated that M4 could prevent the oxidative stress produced by $Aß_{1-42}$ aggregation due to the GM4 samples show similar measures in oxidative stress parameters than the CG without significant difference. There is not significant difference on the $Aß_{1-42}$ levels in the GM4 compared to the GßA, however, GM4 showed lower oligomeric $Aß_{1-42}$ than the others gropus on the westernblot test (WB).

Discussion: M4 could avoid the $Aß_{1-42}$ aggregation, due to lower oligomeric formation showed on the WB results and consequently prevent the oxidative stress induced by oligomeric $Aß_{1-42}$. So, M4 could be a promissory drug candidate to treat AD.

Long term alterations produced on mice prenatally exposed to electromagnetic fields are prevented by trans-resveratrol

Faviola Alcántara-González[1*], Gerardo N. Escalona-Cardoso[1], Roberto Linares Y Miranda[2], and Norma Paniagua-Castro[1]
[1]Department of Physiology, Lab. Development Pharmacology, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Wilfrido Massieu s/n, Esquina Manuel L. Stampa, Colonia Unidad Profesional Adolfo López Mateos, Delegación Gustavo A. Madero, 07738 Ciudad de México, México
[2]Department of Electromagnetic Compatibility, Escuela Superior de Ingeniería Mecánica y Eléctrica, Instituto Politécnico Nacional, Avenida Luis Enrrique Erro s/n, Colonia Unidad Profesional Adolfo López Mateos, Delegación Gustavo A. Madero, 07738 Ciudad de México, México
*Corresponding author, e-mail: falcantarag0800@alumno.ipn.mx, fibi.fibi.2015@gmail.com

Abstract
Built-in Wireless Fidelity (WiFi) devices transmit at a standard frequency of 2.4 GHz and yield electromagnetic fields (EMFs) that increase the oxidative stress in cells. Prenatal exposure to EMFs has been associated with body development alterations, infertility and cellular genotoxicity. The intake of antioxidants during prenatal exposure to EMFs may prevent some of the mentioned alterations. The trans-resveratrol is a natural antioxidant extracted from black grape peel, strawberries, blueberries and peanuts. In this work we tested the preventive effects of trans-resveratrol on the long-term effects of mice prenatally exposed to EMFs. We exposed a group of pregnant mice during gestational days 11 to 17 to a radiation of 2.4 GHz/0.1 W/ 4h per day and with a specific absorption rate (SAR) of 3.0 W/Kg. Another group was exposed to the same radiation but with prior administration of 30 mg/Kg/day of trans-resveratrol. Alterations in body weight at pre-pubertal age, motor behavior in pre-puberty and adulthood, and DNA integrity in leucocytes were prevented in the group of mice prenatally exposed to EMFs with a previous intake of trans-resveratrol. Our data suggest that this antioxidant prevents the alterations displayed in mice prenatally exposed to EMFs.

Effect of $CdCl_2$ on the expression of DNMTs in the HepG2 cell line at subtoxic concentrations

Alcocer Lorenzo Sl* [1], Alarcón Romero LCa[1], Hernández Zavala A_r[2], Hernández Sotelo Da[3], Del Moral-Hernández, O[4], Moreno-Godínez MaE[5], Castro Coronel Ya*[1].

1Laboratory of Cytopathology and Histochemistry, 3Laboratory of Epigenetics of Cancer, 4Virology laboratory, 5Toxicology Laboratory, Autonomous University of Guerrero, Chilpancingo, Guerrero, Mexico. CP. 39090.email: yanethcc@yahoo.com.mx. 2Laboratory of Cellular Morphology. Higher School of Medicine, National Polytechnic Institute. Mexico City, Mexico

Introduction: Cadmium (Cd) is a heavy metal considered an occupational and environmental health problem because it has been classified as a human carcinogen [(1)(2)]. Its effects have been evaluated at high concentrations, however, exposure to subtoxic concentrations with biological relevance in various organs such as the liver has been reported [(3)]. Exposure to Cd has been related to changes in DNA methylation, the effects on methylation machinery such as DNA methyltransferas (DNMTs), enzymes responsible for methylation, have been studied, however, further studies are needed to elucidate the specific mechanisms involved [(4)] [(5)]. **Objective:** To analyze the effect of exposure to $CdCl_2$ on the expression of DNMTs in the HepG2 cell line at subtoxic concentrations. Materials and methods: The cytotoxicity of the concentrations used (0.5, 0.8, 1, and 3 µM) was evaluated through the MTT method in 24, 48, 72 and 96 hours. The level of mRNA expression of DNMTs was evaluated at 0.5, 0.8, 1, and 3 µM for 48 hours through Real Time PCR. **Results:** Exposure to $CdCl_2$ shows a dose-dependent cytotoxic effect, concentrations of 0.5, and 0.8 µM of $CdCl_2$ for 24, 48 and 72 h are not cytotoxic; When evaluating the expression of the DNMTs, a decrease in the expression of DNMT3A is observed by exposure to 0.5 µM of CdCl2 and decreases according to the degree of exposure with respect to the untreated cells. **Conclusion:** The results obtained suggest that exposure to micromolar concentrations of $CdCl_2$ generate a decrease in the expression of DNMT3A.

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New interactions of PPAR gamma with glitazones, 15d-PGJ2 and TZD ring obtained from MD simulations

Samuel Álvarez-Almazán, Feliciano Tamay-Cach, Diana Alemán-González-Duhart, Jessica Elena Mendieta-Wejebe.
Instituto Politécnico Nacional. Escuela Superior de Medicina. Plan de San Luis y Salvador Díaz Mirón s/n, Casco de Santo Tomas, Miguel Hidalgo, Ciudad de México, 11340, México. e-mail: jessmenwej@yahoo.com

Introduction. Type 2 Diabetes Mellitus is a disease caused by a progressive defect in insulin action. Insulin resistance is reduced by different treatments as glitazones. The peroxisome proliferator-activated receptor (PPAR) gamma is glitazones' and endogenous ligands' (15-deoxy-delta-12,14-prostaglandin J2 [15d-PGJ2]) receptor. The aim of this study was to elucidate the interactions presented between those ligands (some with chiral properties) and PPAR gamma.
In a previous study of molecular coupling of some glitazones (2,4-thiazolidinedione [TZD ring], pioglitazone [PIO], rosiglitazone [ROSI] and the (*S*, *S*)-troglitazone [(*S*, *S*)-TRO] enantiomer), similar hydrogen bonds interactions between PPAR gamma and ligands were found. This gave guidelines to perform studies of molecular dynamics in order to find more interactions that could be of importance.
Methodology. Interactions between six crystals of PPAR gamma (PDB: 2PRG, 4PRG, 3T03, 1I7I, and 1FM6) and the endogenous ligand 15d-PGJ2, as well as the 2,4-TZD ring, PIO, ROSI and the (*S*, *S*)-TRO enantiomer were studied using Molecular Dynamics (MD) simulations coupled to molecular mechanics. MD simulations were performed with Particle Mesh Ewald Molecular Dynamics (PMEMD) module AMBER 12 package. The "ptraj" tool in Amber12 was used to analyse the time dependence of the root-mean-squared deviation (RMSD), and clustering analysis.
Results. MD simulations between PPAR gamma and ligands showed similar results. The PPAR gamma-ligand complexes reached stability up to 10 ns (50 ns-long-study), with RMSD values between 1.93 ± 0.22 Å (for 2PRG-15d-PGJ2 complex) and 3.53 ± 0.15 Å (for 4PRG-15d-PGJ2 complex). The complexes between PPAR gamma and ligands were stabilized mainly by hydrophobic residues: Phe226, Pro227, Leu228, Ile281, Phe282, Cys285, Ala292, Ile296, Ile326, Tyr327, Met329, Leu330, Leu333, Met334, Val339, Ile341, Met348, Leu353, Phe363 and Met364. In addition, seven polar residues in helixes H3 and H11 (Arg288, Ser289, Lys367, Gln286, His323, Glu343 and His449) are maintained in the interaction with the receptor.
Hydrophobic residues contributed significantly to the final free Gibbs energy of bind (ΔGbind) value of the stabilization of the each PPAR gamma-ligand complex. Contributions per residue to the final ΔGbind value were due to Arg288 for 15d-PGJ2 and PIO; Cys285, Arg288 and Leu330 for ROSI; Cys285, Arg288, Ile326, Leu330, Leu333 and His449 for (S, S)-TRO isomer; and Phe282 for TZD ring.
Conclusions. Non-polar residues found have been little reported and Glu343 (a polar residue) had not been reported. All these residues in addition with those already reported, can have an important participation in receptor activation and could give crucial information for drug discovery and design of new drugs.

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Antivenom Archeology

Irving Archundia[1], Dayanira Paniagua[1], Felipe Olvera[1] , Anne Wertheimer[2] , Alejandro Alagón[1] , Leslie Boyer[2]
1Instituto de Biotecnología/UNAM, Cuernavaca, Mor; 2VIPER Institute, University of Arizona – Tucson, USA.
Irving Archundia, Instituto de Biotecnología/UNAM, Ave. Universidad 2001, Cuernavaca, Mor. 62210. Tel: (777) 3291602. email: giovanik@ibt.unam.mx

Over many years, the number of exotic snakes has increased in U.S. zoos, universities, museums and private collections, generating a risk, since snakebites occur mostly during manipulation or handling, and severe envenomation, may lead to a fatal outcome. Most of these captive animals are native to Africa, Middle East, Asia and Australia. Although exotic snakebites are extremely rare in the U.S. (about 40 per year), the availability of the proper antivenoms is limited in the U.S. and worldwide. The only specific treatment for envenomation is passive immunotherapy through antivenom administration. Antivenoms are composed of immunoglobulins or their fragments, from plasma of animals that have been hyperimmunized with specific venoms. Their potency is specific against the immunizing venom(s). Cross-neutralization against related species also occurs, but it is not the rule. Consequently, antivenoms specific to captive exotic species should be kept available, when possible, in case an envenomation occurs. Unfortunately for big pharmaceutical companies most of the snakebite global antivenom market has historically been unprofitable, unpredictable and fragmented, hindering investment and resulting in scarcity of approved antivenoms. In many cases medical personnel have elected to use imported antivenoms after their expiration date because in-date material is not readily available from foreign sources. For this reason, our main objective is to evaluate the stability of expired antivenoms still available in the U.S. For this purpose, around 2,500 expired antivenom vials have been collected from zoos, universities, museums and private collections. The initial phase was to classify and prioritize the collected samples. SAIMR products were the first to be analyzed vial by vial. We analyzed 8 vials from 2 lots of monospecific and 73 vials from 21 lots of polyvalent antivenoms. We evaluated the physical stability by visual inspection, and measured their protein using Bradford, A 280nm and BCA assays. For the analysis of qualitative protein composition and integrity of antivenom we used SDS-PAGE under denaturing and non-denaturing conditions. Then, we determined in vitro reactivity against various venoms used for immunization by enzyme-linked immunoassay. Finally, we are measuring their neutralization potencies in the mouse model. Our preliminary results show that all lots have high protein content as compared to current antivenoms; some vials had precipitates of various forms; all vials are composed of high purity F(ab') 2 fragments; some vials had small amounts of soluble aggregates; none of the vials had demonstrable degradation products; ELISA titers are high; and most remain capable of in vivo neutralization of target venoms

Search and evaluation of new molecular structures with potential giardicidal activity

Q.F.B. Juan Carlos Auriostigue Bautista, Dr. Luis Demetrio Miranda, M. en C. Yadira Rufino González, Dr. Jesús Oria Hernández, Dr. Horacio Reyes Vivas. Laboratorio de Bioquímica-Genética, Instituto Nacional de Pediatría; C.P. 04530; Tel: 015510840900 ext. 1442; auriostigue@gmail.com.

The flagellated protozoan *Giardia lamblia* (*G. intestinalis, G. duodenalis*) is the most common human gastrointestinal parasite worldwide. It causes giardiasis, a diarrheic disease that affects mainly children and immunosuppressed people. Current drug therapies include the use of nitroimidazoles, nitrothiazoles, nitrofuranes and benzimidazoles such as tinidazole, nitazoxanide, furazolidone and albendazole, respectively. However, side effects and the emergence of resistance over most commonly used drugs make imperative looking for new antiparasitics through discovering of new biological targets and designing of novel molecules.
Following the pharmacological screening approach, we have analyzed a library of compounds provided by the Instituto de Química, UNAM; with the aim of identifying and evaluating new molecular structures with potential giardicidal activity that can be used for the development of new antiparasitic drugs. For this purpose, an *in vitro* microcytotoxicity assay on *G. lamblia* trophozoites (strain WB) was used, based on a reseeding method and the measurement through tetrazolium salts for cell proliferation and viability. As a first result of this study, three molecular scaffolds have been identified; their structures are totally different from the anti-giardiasic drugs currently used and whose giardicidal activity has not reported before. Briefly, these new structures correspond to a highly substituted benzopyrrolizidine, a bis (aryl ether) macrocycle and a substituted pyrazin-2-one. Analogous compounds with these scaffolds are currently being evaluated to generate lead structures that have better giardicidal activity and low cytotoxicity in mammalian cells.

Cloning, expression and purification of a metalloprotease from the venom of a pit viper endemic of Argentina

Damaris Iyary Bolaños Guadarrama[1], Herlinda Catalina Clement Carretero[1], Ligia Luz Corrales Garcia[2], Adolfo de Roodt3, Gerardo Corzo[1]
[1]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Av. Universidad 2001, Cuernavaca 62210, Morelos, México.
[2]Departamento de Alimentos, Facultad de Ciencias Farmacéuticas y Alimentarias, Universidad de Antoquia, AA 1226, Medellín 050010, Colombia
[3] Laboratorio de Toxinopatología, Centro de Patología Experimental y Aplicada, Facultad de Medicina, Universidad de Buenos Aires, Argentina.
Email: damaris@ibt.unam.mx

Introduction. The snake venoms are complex mixture of proteins components, with different enzymatic and pharmacological activities, the physiological effects of venoms on vertebrates are quite variable. Some poisonous effects produced are hemotoxicity, neurotoxicity and cytotoxicity. *Bothrops ammodytoides* is a small snake of the genus Bothrops endemic of Argentina (Wong and Bolov 2012). Its venom is lethal and causes hemorrhage, dermonecrosis, myotoxicity and edema in mice. It also has procoagulant activity in human plasma (de Roodt et al., 2000). Snake venom metalloproteinases (SVMP) are one of the main enzymes that contribute to the toxicity of bothropic venoms (Fox and Serrano 2005). The metalloproteases isolated from viper venoms could have biotechnological applications in the elaboration of antigens for antivenoms, and also because of its disintegrin domain, which has been used in the therapeutic exploration of different pathologies such as Alzheimer's and autoimmune diseases, inflammation, asthma, osteoporosis, thrombosis and cancer.

Objective. To clone, express and purify a metalloprotease from the venom of the Argentine snake *Bothrops ammodytoides*.

Materials and Methods. Specific oligonucleotides were designed to amplify and to obtain a corresponding metalloprotease transcript.

Results. A metalloprotease was cloned and expressed using *E. coli* as a heterologous system. The resulting recombinant protein was detected by western-blot using anti-histidine antibodies and commercial bivalent antivenoms from Argentine. The recombinant metalloprotease was purified and its molecular mass confirmed.

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Hydroxyurea interferes with the DNA damage response in Fanconi anemia cells.

José Benjamín Bustamante Gómez[1, 2], Bertha Molina[1], Gabriel López V.[3], Sara Frías [1,4 *]
[1]Laboratorio de Citogenética, INP. [2]Doctorado en Ciencias Biomédicas, UNAM. [3]Laboratorio de Bioquímica Genética INP. 4Instituto de Investigaciones Biomédicas, UNAM.
*Torre de Investigación, Instituto Nacional de Pediatría Avenida del IMAN 1, 6o piso, CP 04530 Ciudad de México, Tel. 52 55 1084 1326, correo sarafrias@biomedicas.unam.mx.

Fanconi anemia (FA) is a genetic disease that generates failure in DNA repair and high sensitivity to alkylating agents such as Mitomycin C (MMC). In previous studies we found that FA cells show great chromosomal damage when treated with mitomycin C (MMC) followed by hydroxyurea (HU) applied in G2 phase; HU is an agent that inhibits ribonucleotide reductase (RNR), interferes with replication and activates the FA/BRCA pathway. RNR is composed of two subunits, RRM1 and RRM2 or the variant p53R2; the latter translocates to the nucleus with the help of the p53 protein and positions itself on DNA sites with damage, as a prerequisite for the effective repair of double-strand breaks (DSBs). The aim of this work was to determine if Hydroxyurea interferes with damage signaling and with the participation of ribonucleotide reductase in the DNA repair response after DNA damage, in Fanconi anemia and normal cells. **Methods,** Normal and FA cells were cultured 72 hrs: 1) Without treatment, 2) With HU for 3 h, 3) With MMC for 24 h and 4) Sequential treatment with MMC for 24 h/ HU 3 h. The generation of DSBs and their repair were analyzed in a pulse and 8-chases experiment by flow cytometry for the presence of histone variant ɣ-H2AX in both asynchronous and in G2 phase cells, and the subcellular localization of the subunits RRM1 and p53R2 was analyzed by Western blot and immunolocalization. **Results,** After the induction of DNA damage with hydroxyurea, both FA and normal cells were positive for the generation of DSBs as detected by ɣ-H2AX, followed by an immediate decrease in the subsequent chases, suggesting the presence of alternative repair mechanisms in the FA cells, however in the case of cells treated sequentially with MMC + HU the number of ɣ-H2AX-positive cells was higher in normal cells than in FA, particularly when the cells were in G2, which does not correspond to the high amount of cells with chromosomal aberrations presented by these cells. Western Blot for the p53R2 subunit revealed a marked accumulation of this subunit in the cytoplasm in FA cells, in response to DNA damage, however no translocation to nucleus of this subunit was observed after the damage induction, while this effect was observed in normal cells, where the highest accumulation of p53R2 was found in cells treated with MMC and HU. In the same way in the immunolocalizations of the two subunits of the RNR, a low presence of p53R2 foci was found in the nucleus of FA cells, after MMC-induced DNA damage with or without HU. **Conclusions** The percentage of ɣ-H2AX-positive FA cells after treatment with MMC + HU was not the expected in relation to the high number of cells with chromosomal aberrations that are known to present FA cells treated with both agents, this may be due to cell death, as confirmed by high levels sub-G1 FA cells when treated with MMC+HU; on the other hand, DSBs may be present, but not signaled by ɣ-H2AX, as previously proposed by Rodriguez et al, who observed a dephosphorylation of H2AX by Wip1 in FA cells. Poor translocation of p53R2 to the nucleus in FA cells with DNA damage supports the hypothesis that DSBs are not being repaired in an effective manner when HU is present after DNA damage, but these DSBs are not properly signaled by ɣ-H2AX.

Characterization of proteins with toxic activity from venoms of *Oculicosa supermirabilis, Heriaeus melloteei* and *Dolomedes gertschi*

Samuel Cardoso Arenas, Iván Arenas Sosa and Gerardo Corzo Burguete
Instituto de Biotecnología-UNAM
Av. Universidad 2001, Chamilpa, 62210 Cuernavaca, Morelos
+52 (777) 329-1602

Summary
Spiders are one of the most successful group of poisonous animals in addition to be the most abundant terrestrial predators. One of the reasons for this success is due to the composition of its venom since it has been proven to be a rich source of proteins that cause neurotoxicity through the modulation of ionic channels. In this work we studied the venom of three spiders *Oculicosa supermirabilis, Heriaeus melloteei* and *Dolomedes gertschi*. Their venom proteins were separated by RP-HPLC and evaluated for toxic activity in three animal models: mouse, cricket and fish. Within the venom of *O. Supermirabilis* were detected 45 components from which only two proteins with molecular masses of 6,543.0 and 5,711.2 Da showed toxic activity in crickets, causing paralysis of legs and death. Additionally, a protein of 2,110.4 Da caused just paralysis of legs in crickets but not mortality. Inside the venom of *H. melloteei* were detected approximately 60 components, and a protein of 5,470.9 Da that showed toxic activity against crickets. Finally, the analysis of venom of *D.gertschi* showed approximately 83 components, and three of them with molecular masses of 4,652.2, 5,206.2 and 7,778.8 Da, respectively, showed mortality in zebrafish. Interestingly, we found a protein of 4,815.7Da that showed mortality in mice. The sequence of this peptide suggest that it is a neurotoxin and this is the first report of toxicity of this protein in mice.

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Overexpression of the toxin MCTx-1 from the fire coral *Millepora dichotoma*

Miguel Cuevas Cruz[1,3*], Francisco Miguel García Guerrero y Guerrero[1,2], Ulises Hernández Guzmán[1,4,] Daniel Juárez López[2], Norma Adriana Valdez Cruz[2], Roberto A. Arreguín Espinosa de los Monteros[1*,]
MCC: miguelcuevascruz@gmail.com (56-22-45-16)'RAAE: arrespin@gmail.com (56-22-44-68)
[1]: Departamento en Química de Biomacromoléculas, Instituto de Química, Universidad Nacional Autónoma de México, Circuito
Exterior s/n, Ciudad Universitaria, Coyoacán, Cd. de Mx., 04510.
[2]: Departamento en Biología Molecular y Biotecnología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma
de México, Circuito Mario de la Cueva s/n, Ciudad Universitaria, Coyoacán Cd. de Mx., 04510.
[3]: Programa de Doctorado en Ciencias Biomédicas, Unidad de Posgrado. Edificio B Primer Piso. Universidad Nacional Autónoma
de México, Ciudad Universitaria, Coyoacán, Cd. de Mx., 04510.
[4]: Protein Signaling Program, University of Copenhagen, Nørregade 10, 1165 København, Denmark

Nowadays, the study of marine molecules derived from cnidarian extracts has acquired relevance because evolutionary analysis shows that *phylum* Cnidaria is one of the oldest groups of animals that synthesize bioactive compounds in either defensive or predatory way. Fire coral *Millepora dichotoma* generates a wide variety of proteic toxins among which stand out phospholipases A2 (PLA2), neuropeptides and hemagglutinins; as in the case of the *Millepora* cytotoxin-1 (MCTx-1).
MCTx-1 was the first proteic toxin extracted from the nematocysts of a fire coral, it was purified using size exclusion chromatography and monitored with cytotoxic and emagglutination activity assays with an approximately molecular size of 18 kDa. Once purified, it was sequenced by automated Edman degradation; being the next step the comparative analysis of the primary structure. This way, MCTx-1 was described as belonging to the dermatopontine superfamily. The Dermatopontine was isolated from bovine dermal extracellular matrix, later it was described in humans, pigs, mice and rats; also in invertebrates including crabs, sponges and snails. However, MCTx-1 is the first toxic dermatopontine to be discovered (Iguchi *et al.*, 2008).
From a biomedical point of view, this toxin has great potential because it has cytotoxic activity against mice leukemic cells L1210 (CI50 79 ng/mL)(Iguchi *et al.*, 2008) and it can be tested in human cell lines as in other receptors. MCTx-1 hemagglutinated a 0.8% suspension of sheep erythrocytes (0.2 µg protein/mL) so it has to be tested as well in human erythrocytes and bacteria; the latter, has structural carbohydrates in their walls which can be analyzed by agglutination assays. Crayfish were used to test the LD50and the titre was 106 µg/mL (Iguchi *et al.*, 2008).
Using the Basic Local Alignment Search Tool for proteins (BLASTp) the molecule with the higher similitude was the *Limulus polyphemus* amebocyte aggregation factor (36% of identity and E value $4x10^{-26}$). From the reported dermatopontines, only the *L. polyphemus* aggregation factor and MCTx-1 have hemagglutination activity; however, the first one has PLA2 activity while the fire coral toxin does not, neither does hemolytic activity. It is thought that MCTx-1 takes active participation in the formation of edema associated with the sting of the fire coral *M. dichotoma*.
We used the yeast *Pichia pastoris* to clone the plasmid pPIC9K which contained *mctx-1*, confirmed with PCR, and induced the expression taking advantage of the yeast methylotrophic metabolism. Comparing the production of protein between growing the yeast directly in expression media and growing it in general media then in expression media.
The research of this venom component found in the hydrocoral is limited and the potential utility hasn't been described since the substances produced are used as a tool in the search for New drugs and biotechnological development.

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Effect of phthalates (DBP and BBP) on *in vitro* expansion of human hematopoietic cells

Victor E. Balderas Hernandez, Tomas Ortiz Rodríguez, Antonio de Leon Rodriguez

Instituto Potosino de Investigacion Cientifica y Tecnologica, A.C. Camino a la Presa San Jose 2055. Lomas 4a Seccion. San Luis Potosi. S.L.P. 78216. Mexico. Phone: +52 01 4448342058.
Email: aleonr@ipicyt.edu.mx aleonr@me.com

Abstract

Phthalates are additives used to improve plastic properties such as flexibility and durability, however they are not covalently bound to the polymer matrix, allowing them to easily migrate and pass to human consumption through the diet. In several studies, both *in vitro* and *in vivo*, have confirmed the adverse effects caused by these compounds, from cytotoxicity to endocrine disruption. The human population is continuously exposed to phthalates; because many food products are packaged in plastic containers. These substances have been detected in different human body fluids including umbilical blood cord, which contains hematopoietic stem cells that are used as treatment for many genetic and malignant diseases of the hematopoietic system, primarily in infants. With this the success of therapy might be compromised since it depends on both the quantity and quality of the transplanted cells. The effect of dibutyl phthalate (DBP) and benzyl-butyl phthalate (BBP) on hematopoietic stem cells exposed to these contaminants has not been studied. The aim of this study was to evaluate the effect of two phthalates: DBP and BBP on the *in vitro* expansion of human hematopoietic cells. The results indicate that concentrations, ranging from 0.01 to 100 µg/mL of DBP or BBP, showed cytotoxic effects. A reduction in the total cell expansion was observed, from 28% to 81% for DBP treatment, and from 23% to 68%, for the BBP exposition. Moreover, in the colony forming unit assay, when hematopoietic progenitor were exposed to 10 and 100 µg/mL of DBP, a significant reduction of 74.6% and 99.1%, respectively, was observed. Meanwhile, BBP only showed a negative effect at a concentration of 100 µg/mL. With, these results, we conclude that DBP and BBP might have cytotoxic effects on the hematopoietic cells because they affect their *in vitro* cell expansion. This information is useful because cells exposed to toxic concentrations of phthalates may not be optimal for clinical therapy use. We thank to the partial financial support by the SALUD-2013-01-233340.

Keywords. Plastics, plasticizers, DBP, BBP, endocrine disruptor, toxicity, hematopoietic progenitors, hematopoiesis.

Metformin, Sodium Oxamate and Doxorubicin in combination induce intrinsic apoptosis in human cervical cancer cells

Izamary Delgado-Waldo[1], Rebeca Salgado-García[1], Nadia Jacobo-Herrera[2] and Carlos Pérez-Plasencia[1].

Instituto Nacional de Cancerología, Av. San Fernando No. 22, Col. Sección XVI Delegación Tlalpan, C.P. 14080 México, D.F[1].

Instituto Nacional de Ciencias Médicas y de Nutrición Salvador Zubirán, Unidad de Bioquimica, México, Distrito Federal[2].

Correspondence author: Av. San Fernando No. 22, Col. Sección XVI Delegación Tlalpan, C.P. 14080 México, D.F. carlos.pplas@gmail.com

Cervical cancer (CC) is a public health concern. In Mexico it is the second leading cause of death by cancer behind breast cancer in women, with 13 960 new diagnosed cases every year and 4 769 deaths in 2015. In most cases, CC originates in the stratified epithelium, from the uncontrollable growth of cells: since 1977, it is known that the etiological factor responsible for 99.7% of this neoplasm is the human papillomavirus (HPV), which induces changes in cellular functions that favor the replication of viral particles and the transformation of normal cells to cancer cells favoring processes like high proliferation, apoptosis resistance. Previous studies have shown that cervical cancer progression can be associated with deregulated cellular energetics known as the Warburg effect. This alteration plays an important role in tumor progression as it provides essential energy for cell proliferation and tumor growth; therefore, it has shown great potential as a therapeutic target. In this work, we used a combination therapy aimed to disrupt tumor cell energy pathways: metformin –an inhibitor of the electron transport chain complex I– and sodium oxamate –an LDH-A inhibitor– in combination with doxorubicin, the standard treatment for many types of cancer. We estimated inhibitory concentration (IC_{50}) in combination therapies. Our results showed that the triple therapy suppressed proliferation in an *in vitro* model. We next assessed cell death through flow cytometry; cells exposed to the triple therapy showed early apoptosis. Finally, we detected the expression of apoptosis-related gene products by protein microarray and western blot analysis. Our results provide useful clues for targeting deregulated cellular energetics in cervical cancer through metabolism inhibitors and triggering apoptosis.

Key words. Cervical cancer, Intrinsic Apoptosis, Warburg effect, metformin, sodium oxamate, and doxorubicin.

"EFFECTS OF ATRAZINE ON THE HUMAN TROPHOBLAST"

Dr. Domínguez López Pablo E., Dra. Álvarez Arellano Ma. De Lourdes.
UIMMR Hospital de Ginecología y Obstetricia No. 4 "Dr. Luis Castelazo Ayala" IMSS.
Av. Rio magdalena No. 285 col. Tizapan San Angel c.p. 01090 Ciudad de México.
p.enriquedominguez@gmail.com Tel. 55506422 ext. 28039.

Introduction: The pesticide Atrazine belongs to the 2-chloro-s-triazine family of herbicides. It is one of the most commonly used herbicides worldwide, has been detected as one of the main pesticide contaminants of ground-water and surface water. In Mexico, the use of atrazine is not regulated, and it is being widely used in agricultural areas. The Mexican regulations on water quality provide limits for some pesticides, but these do not include limits for atrazine, possibly this is the reason why the concentration of this herbicide has been detected above the recommended limits for drinking water. From this perspective, we can affirm that the arbitrary and indiscriminate use of atrazine in the areas of agricultural cultivation, represents a great risk of contamination of the aquifer mantles.
During pregnancy, the endocrine disrupter chemicals (EDCs), as Atrazine, have adverse effects on human placenta. It has been shown that atrazine decreases the synthesis of hCG hormone in human trophoblastic cells. These cells are in direct contact with both maternal blood and tissues and, therefore, are greatly exposed to environmental polluting chemicals which gain access to maternal blood. The placenta is the foremost organ in establishment and maintenance of pregnancy as well as for fetal growth and development. Nowaday known to activation of inflammation and oxidative stress pathways, is strongly associated with fetal growth restriction. Therefore, it is important to evaluate the potential toxicity of environmental polluting chemicals in order to define the risks to human health during pregnancy.
Objective: The present study was designed to determine the effects of atrazine in vitro, on human trophoblast, using the trophoblast-derived choriocarcinoma BeWo line cell.
Material and methods: The choriocarcinoma-derived BeWo cell line ($1.5X10^3$/well) were seed into 96-well microplates for the viability test using comercial CytoTox 96 non-radioactive cytotoxicity assay kit.
To determine effect of atrazine on human trophoblast, ($5X10^4$/well) were seed into 24-well plates containing 1 mL of DMEM culture medium without FBS supplemented with the atrazine at concentrations ranging from 10 picomolar (pM) to 100 micromolar (μM), for 72 hours. The cellular media were collected for subsequent TNF-alpha and TGF-beta, IL-10 and IL-1beta cytokine determination by ELISA.
Results: The exposure of trophoblasts to different concentrations of atrazine (pM–μM), did not affect cell viability. We observed at μM and pM concentrations of atrazine, the production of TNF-alpha and TGF-beta cytokines increases, whereas when we use an intermediate concentration range of atrazine from 10 to 100 nanomolar (nM), the expression of the two cytokines decreased significantly. With respect to IL-10 and IL-1beta cytokines, atrazine had no effect on the synthesis of these.
Conclusion: The present study brings to the light important effects on human trophoblast exerted by environmental polluting chemicals. Atrazine there was a biphasic effect on the production of cytokine TNF-alpha and TGF-beta in the human trophoblast. Is interesting since this imbalance can alter the processes of implantation and placentation in pregnant women exposed to the herbicide and potentially, contribute to the development of gynecological and obstetric pathologies such as intrauterine growth retardation and preeclampsia. The effects sustained at concentrations as low as pM raise great concern about the environmental risk to pregnancy, pointing to the need for protecting both pre-natal life and development of the foetus.

Effects of extract of *Lepidium virginicum* on DNBS-induced colitis in rats

María Fernanda Durán Vázquez, Tonatiuh Barrios García, Eduardo E. Valdez Morales, Sugela Susana Blancas Sugarazo, Yenny Adriana Gómez Aguirre, *Raquel Guerrero Alba
Universidad Autónoma de Aguascalientes, Centro de Ciencias Básica, Departamento de Fisiología y Farmacología. Av. Universidad # 940. Colonia Ciudad Universitaria. C.P. 20130. Tel (449) 9 10 7400 ext 51261 o 8407. *correo electrónico raquel.guerrero@edu.uaa.mx

Background and aim: Ulcerative colitis (UC) is an idiopathic inflammatory bowel disease characterized by an abnormal activation of the immune system associated with the gut, resulting in a chronic inflammation of the rectum and colon. The major symptoms of ulcerative colitis include diarrhea, abdominal pain, gastrointestinal bleeding, weight loss and variable periods of activity and recurrence[1]. Corticosteroids and opioids are currently used for the treatment of this disease. However, the adverse side effects and/or lack of full effectiveness are common[2]. Therefore, traditional medical plants may be an important alternative effective without side effects. *Lepidium virginicum L.* is an important medicinal plant from *Brassicaceae* family originated from USA and Mexico. It is traditionally used as alternative herbal medicine for gastrointestinal illness including abdominal pain and dyspepsia. Additionally, it has been reported that *L. virginicum´s* extracts contain many bioactive compounds as phenolic acid (gallic acid), flavonoids (quercetin), terpenes, sterols, coumarins, which are directly related to possess anti-inflammatory and/or antioxidant properties[3]. So, these properties of *Lepidium* suggesting that it may has a high therapeutic potential for UC conditions. Therefore, the aim of the present study is to evaluate the effect of extract of *L. virginicum* in an animal model of experimental colitis. **Methods:** Stems of *L. virginicum* were collected from the campus of Aguascalientes Autonomous University during March 2018. After collecting and identifying, the plant was air-dried; 500 g of stems was finely powdered, wetted by 95% of ethanol and extracted using percolation apparatus. The extract was shaken, filtered and evaporated in a rotary evaporator under reduced pressure until a semisolid extract was obtained. Test dose was eventually prepared by reconstitution of this dried extract. Wistar rats were randomly assigned in four groups of at least 6 rats as following: 1) Sharm group, treated with vehicle without colitis; 2) control group, treated with extract without colitis 3) Colitis group, without treatment 4) Extract group, treated i. p. with *L. virginicum extract* at dose of 100 mg/Kg after induction of colitis. Colitis was induced by intrarectal administration of Dinitrobenzene sulfonic acid (DNBS) 200 mg/ Kg in ethanol 50% v/v. To determine the severity of clinical colitis, body weight, stool consistency and visible fecal blood were scored. **Results:** Rats treated with DNBS showed significant weight loss, colon shortening, diarrhea and visible fecal blood. In contrast, rats treated with ethanolic extract of *L. virginicum* for 6 days after colitis induction showed significant attenuation of these phenotypes. **Conclusion:** These results suggest that extracts of *L. virginicum* attenuates the pathologies of DNBS-induced colitis.

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Determinación de la actividad de una fracción menor a 1 kDa del veneno de *Palythoa caribaeorum* (Duchassaing & Michelotti, 1860) en el canal iónico Kv10.1

Noel Fabian-Hernández, Miguel Cuevas-Cruz, Enoch Luis-Baltazar, Arturo Picones-Medina, Arturo Hernández-Cruz, Judith Sánchez-Rodríguez, José Fernando Lazcano-Perez, Sergio Román-González & Roberto Arreguín-Espinosa.
Instituto de Química, Circuito Exterior s/n, Ciudad Universitaria. Delegación Coyoacán, C.P. 04510, México, D.F. arrespin@gmail.com

Kv10.1 ion channel is over expressed in most human solid tumors (70%) and contributes to metastasis and disease progression (in fact, Kv10.1 channel is considered as indicator of cancer aggressiveness), making this channel suitable target for cancer treatment. The small peptides capable of blocking ion transport through this gate are thought as candidates for cancer therapy (Stühmer, 2017) and so far, only K-hefutoxin (3034.7 Da) from the venom of *Heterometrus fulvipes* and APETx4 (4650.99 Da) from the venom of *Anthopleura elegantissima* have shown activity towards Kv10.1 with a inhibitory activity of 57±2% and 76±2% respectively (Moreels *et al.* 2017b).
A low molecular weight fraction (< 1 kDa) isolated from the venom oh the zoanthid Palythoa caribaeorum, showed an inhibitory activity over the potassium currents and specially over the Kv10.1 ion channel, measured by “patch clamp” technique in the modality of complete cell with an inhibitory activity up to 60% at 1 $\mu g \cdot mL^{-1}$. The fraction aforementioned was purified through ultrafiltration and RP-HPLC.

Molecules with inhibitory activity over Kv10.1 channel had been reported previously in *H. fulvipes* and *A. elegantissima (*Moreels, *et al., 2017a)*, with molecular masses between 3 and 4 kDa that differ from Palythoa. Based on our results, we suggest that some of the components of the active fraction could be used as therapeutic agents for the treatment of various types of solid tumors.

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Indomethacin: As posible selective inhibitor of phospholipase A2.

Leticia Guadalupe Fragoso Morales[1,2] Martha C. Rosales Hernández[2] José Correa Basurto[1] *

Instituto Politécnico Nacional, Plan de San Luis y Díaz Mirón s/n, Casco de Santo Tomás, Distrito Federal 11340, México; Tel.: +55 57296000 ext. 62809 Correspondence: corrjose@gmail.com* marcrh2002@yahoo.com.mx

Introduction:
Phospholipases A2 are a large family of enzymes that hydrolyze the glycerophospholipid in the sn-2 position to produce a free fatty acid and a lysophospholipid, however, the increase in the generation of lipid mediators such as arachidonic acid, lysophosphatidic acid and diacylglycerol. [1]
To date, there are different terapeutic inhibitors for each of the phospholipases A2, however, they have not been used as a treatment.
Nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin are well known for the treatment of several diseases, such as arthritis, fever and pain. The pharmacological effects of these drugs are largely attributed to the inhibition of prostaglandin synthesis mediated by cyclo-oxygenase (COX). [2]
Knowing that the pharmacological effect of indomethacin is largely attributed to the inhibition of prostaglandin synthesis mediated by cyclooxygenase (COX), the inhibition of PLA2, which hydrolyzes the lipids of the cell membrane, should not be ruled out.
Objective: To analyze in silico and in vitro the possible selective inhibition of PLA with indomethacin
Methodology: To *in silico* study was used molecular docking using the three differents proteins, however was used NCBI to download the PDB'S and to achieve *in vitro* study was used EnzChek Phospholipase A2 Assay Kit" Invitrogen.
Results and discussion
With the *in silico* evaluation we observed an interacction favoreced to a secretory phospholipase A2
Regarding *in vitro* evaluation, indomethacin was used, which has been reported as a PLA2 inhibitor in human synovial fluid (IC 50 of 35 μM). However, the IC50 that has been obtained from the kit was 663.99 μM.
Conclusion.
With this study we can conclude that Indomethacin reduced of PLA2 activity and could be as a selective inhibitor.

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Functional expression of non-CB1 and non-CB2 cannabinoid receptor in leukemia cell lines.

Pamela Gaitán González [2], Arturo Daniel Villanueva Magaña [2], Esmeralda Espinoza Valencia [1], Luis Marcelino Ramírez Maldonado [2], Erick Giovanni Bello Mendoza[2], Andrómeda Liñán Rico[2], Oxana Dobrovinskaya2, Adán Dagnino Acosta2, Georgina Valencia Cruz2, Edgar Octavio Bonales Alatorre[2] ebonales0@ucol.mx
(1) Facultad de Ciencias Químicas, Universidad de Colima, Km. 9 Carretera Colima-Coquimatlán, Col., México 28400. (2) Centro Universitario de Investigaciones Biomédicas, Universidad de Colima, Av. 25 de Julio # 965, Col. Las Víboras, C.P. 28040, Colima, Col.

There have been more than 700 genes identified that encode G protein-coupled receptors (GPCRs), representing by far the largest protein superfamily involved in signal transduction of the human genome. GPCRs detect a remarkable variety of signals and play key roles in mediating a wide variety of biochemical functions involved in key physiological processes including neurotransmission, immune responses, cardiac- and smooth-muscle contraction, and hormone and enzyme release, to name a few. Over 40% of all FDA-approved drugs are aimed at targeting GPCRs or their related pathways. Evidence demonstrates that signaling through GPCRs affects numerous aspects of cancer biology such as vascular remolding, invasion, and migration.

Cannabinoids, the active ingredients in marijuana, have dramatic effects on various organ systems. They mainly exert their effects through two receptor types: CB1, primarily located in the brain, and CB2, primarily located in the immune system. Interestingly, some of their effects cannot be explained by the signals through either CB1 or CB2. These receptors are not activated by abnormal cannabidiol (abn-CBD); nevertheless, abn-CBD acts as a selective agonist at the orphan receptors GPR55 and GPR18, which are expressed in various cancer types in an aggressiveness-related manner and have a critical role in regulating cancer-cell proliferation. Although these two orphan receptors are overexpressed in leukemia cell lines, their impact in the regulation of the intracellular calcium network has not been systematically explored.

Calcium, the universal second messenger, is a highly versatile intracellular signal that operates over a wide temporal range to regulate many different cellular processes. It has a direct role in controlling the expression patterns of its signaling systems that are constantly being remodeled in healthy as well as diseased cells. In this work we evaluated the calcium mobilization response to abnormal cannabidiol of two leukemic cell lines (REH; B cell leukemia and CEM; T cell leukemia). Records of intracellular calcium concentration [Ca2 +]i in cell populations labeled with FURA2-AM and measured by spectrofluorometry, showed a concentration-dependent increases in [Ca2+]i in the presence of 10, 15 and 30 uM abn-CBD, besides. The cell populations displayed a different sensitivity to the component according to each cell line. The rise in the [Ca2 +]i was considerably blocked by 10uM of CID16020046, a specific GPR55 agonist.

These results show that the contribution of functional GPR55 receptors (without discarding GPR18 ones) could be an important mechanism for the calcium regulation network of leukemia cells. This opens the possibility for the identification of G-proteins' new signaling pathways and physiological functions, which needs to be further interrogated in order to develop more efficient cancer biomarkers and anti-leukemic therapies.

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Extracts of *Capsicum annum L* modulate the metabolic activity of MCF-7 and MCF-12 cell lines.

Roberto Jorge García Mendoza[1], Verónica Morales Tlalpan[1,2], Jorge David Gomez[2], Angelica Feregrino-Pérez AA[3], Adriana González-Gallardo[4], Saldaña C[1].
[1]Facultad de Ciencias Naturales, [2]Facultad de Medicina, 3Facultad de Ingeniería. Universidad Autónoma de Querétaro. 4Unidad de Proteogenómica. Instituto de Neurobiología. Universidad Nacional Autónoma de México. Roberto Jorge García Mendoza, Dirección postal: calle 15, no. 810, col. Lomas de Casa Blanca, Querétaro, Querétaro. Cp.: 76080. Teléfono: 4426140575, correo electrónico: biotouhou@gmail.com

Breast cancer affected between 2003 and 2010 more than 4.4 million women in the world. Efforts to understand the molecular mechanisms underlying this pathology are still under investigation.
The phytochemicals present in fruits, vegetables, grains, spices and teas have been the subject of various investigations; because they have been shown to modulate the onset, promotion, progression and metastasis of cancer. The production of certain phytochemicals of *Capsicum annuum L* can be artificially enhanced in greenhouses. In crops *C. annuum L*, under certain conditions the production of capsaicin and its derivatives can be activated. Capsaicin can inhibit viability and tumorigenesis in bladder cancer cells and in small lung cancer cells.
In this work, we show the effect of pepper extracts *Capsicum annuum L* from 3 varieties: Orangela (ORA), Baselga (BAS) and Fascinatum (FAS), in two human breast epithelial cell lines, MCF-7 (cancerous), and MCF-12F other non-cancerous. Our results show that the metabolic activity, determined by the oxidation of resasurin to resofurin, in response to ORA, BAS and FAS, is differentially modulated by these extracts. The extracts ORA and FAS increased the metabolic activity in both cell types, interestingly, the BAS extract induced a reduction in the metabolic activity in both clones.

Prevalence of polymorphisms in the human UDP-Glucuronosyl transferase UGT1A6 552A>C (Arg184Ser) in mexican mestizo population of the state of Puebla.

Wendy Argelia García Suastegui1*, Katty Meritxell Sánchez Sánchez2, Anabella Handal Silva1, José Luis Morán Perales1, Miguel Calva Maldonado3, Silvia Zepeda Olivera3, María Eugenia Gonsebatt Bonaparte4.
1Departamento de Biología y Toxicología de la Reproducción, Instituto de Ciencias, 2Licenciatura en Biotecnología, Facultad de Ciencias Biológicas, 3Hospital Universitario, Benemérita Universidad Autónoma de Puebla, 4 Sur 104, Col. Centro C. P. 72000. Teléfono 2295500 Extensión 5649.4Departamento de Medicina Genómica y Toxicología Ambiental, Universidad Nacional Autónoma de México.
*corresponding autor: wendy.garcias@correo.buap.mx

Abstract

UGT1A6 is a phase II metabolism enzyme that catalyzes the glucuronidation of widely used drugs. Interethnic differences in genetic polymorphisms of UGT1A6 have been reported; however, there are no reports of their frequencies in mexican population. The aim of this work was to determine the frequencies of UGT1A6 552A>C (Arg184Ser) in a mexican mestizo population of the state of Puebla and compare them with the frequencies observed in other populations. Peripheral blood was obtained from 80 healthy adults to extract DNA and identify UGT1A6 alleles by PCR-RFLP assay. The most common genotypic frequency was the wild type homozygous (A/A) 77.5%, followed by the heterozygous (A/C) with a frequency of 22.5%, homozygous mutant individuals were not observed (C/C).When comparing the results of our research with those observed in other ethnic groups, we observed that the UGT1A6 mutant allele frequencies were higher than those reported in the Portuguese population and lower than those reported in Japanese, Hindu, Chinese and Greek populations. These results must be considered to make decisions when choosing the drugs administered to different ethnic groups.

Acute intoxication with sodium nitrate produces hematological and biochemical alterations and expression of hypoxia response genes

*Rebeca Pérez Morales[1], María Fernanda González Delgado[1], Alberto González Zamora[2], María Eugenia Gonsebatt Bonaparte[3].
[1] Facultad de Ciencias Químicas. Universidad Juárez del Estado de Durango.
[2] Facultad de Ciencias Biológicas. Universidad Juárez del Estado de Durango.
[3] Instituto de Investigaciones Biomédicas. Universidad Nacional Autónoma de México.
*Autor responsable. Rebeca Pérez Morales. Laboratorio de Biología Celular y Molecular, FCQ-UJED. Av. Artículo 123 s/n. Fracc. Filadelfia. C.P. 35010. Gómez Palacio Durango. Tel (871) 7 15 88 10 ext. 128 e-mail rebecapms@ujed.mx.

Introduction. Nitrate pollution is caused by excessive soil fertilization and inadequate management of industrial and domestic wastewater. Nitrates are reduced to nitrites within the organism and promote the transformation of hemoglobin into methemoglobin and the formation of reactive species. Under conditions of methemoglobinemia the cells must adapt to hypoxia and oxidative stress. The aim was to analyze the effect of acute $NaNO_3$ intoxication in male Wistar rats.

Materials and methods. Wistar rats of 2-3 months of age and a weight between 100-150 g were administered during 10 days with 19, 66 and 150 mg/Kg of $NaNO_3$. The determination of metaHb and NO_2/NO_3 were carried out by spectrophotometry. Regarding the determination of hematological and biochemical parameters were carried out in automated equipment. For the determination of oxidant stress markers Cayman kits were used. Histological samples were obtained to analyses of tissue, and for expression assays were carried out using Taqman probes.

Results. High percentages of metaHb with a dose response effect were found. In the hematological parameters increased the number of white blood cells; while in the biochemical parameters significant differences were found in glucose and triglyceride levels. In addition, an increase in liver enzyme levels was found in the plasma of the group treated with 150 mg/kg of $NaNO_3$. We also found an increase in the activity of LDH and SOD, in addition to an increase in the total GSH. In the treated groups, alterations in the mitochondrial function and the increase in the antioxidant response correlated with the levels of nitrosylation found at high doses. The previous findings agree with morphological changes in the liver tissue of the treated groups in which inflammation, steatosis and necrosis were found. Immunolocalization of Hif1a and Hif2a was performed and reactivity in the nuclei of the liver tissue was found, indicating the stabilization of the transcription factor. Regarding gene expression, the genes with hypoxia response elements *Ldha, Sod2*, *Vegf, eNos* were modulated.

Conclusion. The results show that acute intoxication with $NaNO_3$ generates a condition of methemoglobinemia and oxidative stress that induces an adaptive response through metabolic reprogramming and the antioxidant response. These changes are promoted through the activation of the Hifs; however, in high concentrations of $NaNO_3$ (66 and 150 mg/kg) the cell loses its adaptive capacity establishing cellular damage in the liver tissue.

Toxicological Evaluation of Textile Wastewater (Denim process).

Anabella Handal Silva*, Andrés Estrada Rivera, Fabiola Avelino Flores, Wendy Argelia García Suastegui, Fernando Hernández Aldana, Octavio Sánchez García, José Luis Morán Perales. Benemérita Universidad Autónoma de Puebla, Posgrado en Ciencias Ambientales del ICUAP. 4 Sur 104, Col. Centro C. P. 72000. Teléfono 2295500 extensión 7161. Correo ahandals@yahoo.com.mx

Abstract.
This work is aimed at evaluating toxicological damage in fish, due to the effects of textile waste water (Denim process), which are discharged to the Atoyac River in the town of Villa Alta, Municipality of Tepetitla de Lardizabal Tlaxcala. The textile industry is one of the main generators of hazardous pollutants, air emissions, solid and liquid waste, energy consumption and the generation of large volumes of highly colored wastewater composed of hardly biodegradable compounds. Our objective was to evaluate the toxicity of textile wastewater, and its effect on zebrafish morphology and physiology. The samples of textile wastewater (TWW) were obtained according to Official Mexican Standard NMX-AA-003 and its physicochemical analysis were performed according to NOM-001-SEMARNAT-1996. The NOM-062-ZOO-1999 was considered for the care and use of laboratory animals. The bioassays were carried out for 96 hours, by triplicate, according to OECD 502 guidelines. The composition of the TWW was analyzed. Bioassays were performed using zebrafish as biological model exposed to different concentrations of TWW (75, 50, 40, 35, 25, 15 and 13.5%). The zebrafish gills and liver were analyzed histologically. The results showed that seven TWW parameters (total nitrogen, sedimentary solids, fats and oils, BOD_5, phosphorus, COD, total sedimentary solids) are outside the NOM-001-SEMARNAT-1996, their volumes discharged into the river were also determined. The estimated quantity of pollutants poured into the Atoyac River was 31.2 tons/month. The TWW flow was 3.6 L/s. the LC_{50} of the TWW was 19.04%. It was observed, over the fish body surface, an increase in the mucus secretion and muscular softening in the 40-75% concentrations. The histological analysis of gills showed alterations in the lamellar symmetry of the branchial filaments, as well as the thickness of its epithelia, this alteration results in the dysfunction of the respiratory organ. The liver tissue of the fish showed a normal appearance, with a uniform coloration. It is concluded that TWW, has adverse effects on the ecosystem and on fishes. It is important to mention that works of this kind are limited in the study area. This research was carried out with the support of CONACYT and the Vice-Rectory for Research and Postgraduate Studies of the Benemérita Autonomous University of Puebla.

Gestational exposure to particle matter and genic expression changes of enzymes related with Polycyclic Aromatic Hydrocarbon metabolism and DNA repair.

C. V. Huitrón-Román, J. A. Maciel-Ruiz, R. Robles-Morales, M. G. Veloz-Martínez, P. Petrosyan, P. Ostrosky-Wegman, M. E. Gonsebatt.

Particulate matter (PM) is a mixture of liquid and solid particles, which contain a wide variety of compounds including polycyclic aromatic hydrocarbons (PAHs). PM and some of the PAHs are cataloged as carcinogens by the International Agency for Research on Cancer (IARC). Both pollutants are present in Mexico City Metropolitan Area (MCMA) air pollution. PAHs enter the human body through the respiratory and digestive systems. Their hydrophobic character allows them to enter the cells passively, where they are metabolized by the cytochromes P450 CYP1A1 and CYP1B1 to be eliminated. However, during this process reactive species are generated which can react with DNA and trigger genotoxic damage that could lead to cancer.

Exposure to air pollution is related to health adverse effects in newborns, and associated with low fetal growth, endocrine disruption and decreased intellectual coefficient. The identification of molecular markers to measure the impact of PM in placenta, can help us uncover mechanistic information to determine possible adverse health effects associated with air pollution and identify the gestational windows with higher harmful effect associations.

CYP1A1 and *CYP1B1* gene expression is not constitutive, their transcription is modulated by PAHs that bind to the transcription factor AhR. Here, we investigate the transcription of *CYP1A1* and *CYP1B1, AhR* and the DNA repair genes, *MGMT* and *ERCC1* in human placenta as biomarkers of gestational exposure to air pollution in the MCMA. Thus, we measured gene expression by quantitative real time PCR in human placentas collected at the Hospital de Gineco-Obstetricia 3 in La Raza Centro Médico in the MCMA during 2014-2015 and associated their expression with PM levels reported by the Automatic Atmospheric Monitoring Network of Mexico City (RAMA) and with the levels of DNA damage in cord blood.

Arsenic distribution in 21 High Schools in Guanajuato State

Varinia López-Ramírez[1], Isabel Rodríguez-Meza[3], Jan Roelof Van Der Meer[2] and Cesar Álvarez-Mejía[3].

[1]Coordinación de Ingeniería Bioquímica. Instituto Tecnológico Superior de Irapuato, Irapuato, Gto; [2]Department of Fundamental Microbiology, University of Lausanne, 1015 Lausanne, Switzerland; [3]Coordinación de Ingeniería Ambiental, Instituto Tecnológico Superior de Abasolo, Blvd. Cuitzeo de los Naranjos No. 401. Abasolo, Gto. C.P 36976, email: cesar.alvarez@tecabasolo.edu.mx, phone (429)1061300 ext. 1026.

Arsenic pollution in water is related to a geological source, and is increasing nowadays. Due to drinking water scarcity, water extraction for human consumption has been explored as deep as 1500 meters in Guanajuato State. Therefore, the quality for drinking water has been compromised and the presence of minerals such as calcium, magnesium and carbonates and water's hardness had been increased. Fluoride and arsenic have been simultaneously detected in the composition of the groundwater, with values above the Mexican Official standards (NOM-127-SSA-1994). People that consume this water without treatment exhibit some symptoms of intoxication, its chronic ingestion of polluted water has been associated with cancer, skin lesions like hyperkeratosis, cardiovascular problems and direct effect on the IQ of children, diabetes and recently epigenetics changes. Our group is interested in arsenic detection in water sources and also in the design of strategies of water purification and the evaluation of arsenic effects in the exposed population. For that reason, we use a biomonitoring system to detect arsenic in 21 High School water samples, using *lac*Z as gene reporter, the intensity of blue color is proportional at arsenic concentration, the minor As quantity detected for this system is closer to 25 ppb, that is the maximum limit permissible (LMP) for As in water. From the different intensities of color, there were reported as in the limit or non-arsenic presence when the blue color is almost visible or is absent, increasing the blue color were taken as above to the LMP and considered as a risk to As intoxication. The schools evaluated were CECyTE (Colegio de Estudios Científicos y Tecnológicos del Estado de Guanajuato). We found that 28.57 % of CECyTE have high concentration of arsenic, 47.61 % are closer to the NOM highest limit, 19.04% are in the limit or lower. The CECyTE distribution were mapped and analyzed to detect zones in Guanajuato State that increased the arsenic presence in water and the chances to be related to hydrologic scenario. These results show an arsenic risk intoxication map, the proximity of students expose to this pollutant. We advise to the Guanajuato state to verify the purification system and warn at the potential exposed population not to drink water from tap.

Fraccionamiento e identificación de la actividad proteolítica del veneno de *Palythoa caribaeorum*(CNIDARIA:ANTHOZOA: ZOANTHARIA).

Martha Mayela Manzano Mora[1], Alan G. Hernández-Melgar, Miguel Cuevas-Cruz, Sergio A. Román-González, Roberto A, Arreguín-Espinosa de los Monteros[1] & Barbarín Arreguín-Lózano[1].
MMM: mayela.mora94@gmail.com RAE: arrespin@unam.mx
[1] Instituto de Química, Universidad Nacional Autónoma de México. Circuito Exterior s/n, Ciudad Universitaria. Delegación Coyoacán. C.P. 04510, México D.F. Teléfono:+52 (55) 56 22 44 20.

Palythoa caribaeorum is a broadly distributed Zoanthid species due to factors such as the colony phenotypic plasticity, phisiological tolerance and anti-depredatory mechanisms like toxin (palytoxin) production which accounts for its label as an aggressive territorial competitor antidepredatory (Acosta *et al.* 2001; Rabelo *et al.* 2013). It is well known that Cnidarian poison is a complex mixture of molecules like peptides, proteins, enzymes and proteases inhibitors (Frazão 2012). However, very few proteases have been isolated from marine venoms (Cnidarians included). Therefore, the present work aims to determine the proteolytic activity from the venom of *P. caribaeorum.*

Proteolytic activity was evident in different fractions from *P. caribaeorum* venom (molecular masses of 75 KDa and greader than 245 kDa). In addition, of the 7 fractions obtained from the recirculation of FC in molecular exclusion, the proteolytic activity was determined by the method of Buroker-Kilgore & Wang (1993), where 4 fractions showed activity greater than 20%. The protein, identification trough matrix-assisted laser desorption/ionization (MALDI) tandem mass spectrometry was not carried out due to the lack of sequences of proteases in cnidarians databases.

Keywords: *Palythoa caribaeorum*, proteases, zymogram, cnidarians, venom.

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Erythropoietin reduces collagen deposition and attenuates renal fibrosis in an experimental model of Chronic Kidney Disease

Ana Laura Márquez-Aguirre [1]*, Estefanía Vázquez-Méndez [2], Yanet Gutiérrez-Mercado[1], Claudia Morales-Martínez[1], Hugo Esquivel-Solís H[1], Francisco Javier Gálvez-Gastélum[2]
[1]Unidad de Biotecnología Médica y Farmacéutica, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C., Guadalajara, Jalisco, México.
[2]Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Jalisco, Mexico.
C.P. 44720, +52(33) 33455200 ext. 1670, amarquez@ciatej.mx

BACKGROUND: Chronic kidney disease (CKD), is characterized by a persistent inflammation, leading to fibrosis and loss of renal function. Human Recombinant Erythropoietin (rHuEPO) has been widely used to treat CKD-associated anemia and is known to possess organ-protective properties that are independent from its well-established hematopoietic effects.

METHODS: In this study, CKD was induced by oral administration of 100 mg/kg/day of adenine during four weeks. Male Wistar rats were treated with rHuEPO administered subcutaneously 1050 IU/kg once weekly. Control groups were orally administered with vehicle and treated with placebo. Urinary volume, serum creatinine (SCr) and urea as well as hematocrit, erythrocyte count and hemoglobin (Hb) levels were monitored to assess renal function and anemia, respectively. The effects on renal tissue were evaluates by morphological (H&E) and immunofluorescence methods for α-smooth muscle actin (α-SMA) and collagen type 1 (Col 1α).

RESULTS: A decreased kidney function and anemia were found in model control group -or adenine-feeding rats- (CDK). Adenine feeding caused significant decrease in body weight and a significant increase urine output, SCr and urea accompanied by decreases in Hb, hematocrit and erythrocyte count. Remarkable histological changes of kidney tissues characterized by inflammatory infiltrate, interstitial fibrosis and tubular dilatation were observed in CDK. As well, an increased α-SMA and Col 1α deposition. In contrast, rHuEPO-treated rats, decreased SCr, urea and increased Hb, erythrocyte count and reticulocytes promoting anemia correction; while the kidneys revealed less tubulointerstitial fibrosis and fewer collagen deposition.

CONCLUSION: Our data seem to suggest a potential role of erythropoietin for reducing the progression of fibrosis in an experimental rat model of CKD. This protective effect is, in part, could be attributable to anemia correction.

Cacalol acetate as inhibitor of the NF-κB pathway

Beatriz Mora Ramiro[1], Rosario Wendoline Rosiles Alanis[1], Luis Enrique Gómez Quiroz[2], José Luis Ventura Gallegos[3], Alejandro Zentella Dehesa[3], Julio Cesar Almanza Pérez[1].
[1]Lab. Farmacología, Depto. Ciencias de la Salud, Universidad Autónoma Metropolitana-Iztapalapa. San Rafael Atlixco 186, Vicentina, Iztapalapa, C.P. 09340, México, CDMX.
[2]Lab. Fisiología Celular, Depto. Ciencias de la Salud, Universidad Autónoma Metropolitana-Iztapalapa. San Rafael Atlixco 186, Vicentina, Iztapalapa, C.P. 09340, México, CDMX
[3]Unidad de Bioquímica, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. Avenida Vasco de Quiroga No.15, Colonia Belisario Domínguez Sección XVI, Delegación Tlalpan C.P.14080, México, CDMX.
jcap@xanum.uam.mx

Many diseases are characterized by inflammatory processes such as multiple sclerosis, rheumatoid arthritis, cancer, diabetes, among others. The transcription factor NF-κB is one of the main therapeutic targets in these diseases, since the binding to its consensus sequence induces the transcription of important inflammatory mediators (TNF-α, IL-6, IL-1β, COX-2). There are several alternatives in the pursuit of new therapeutic substances, one of them are medicinal plants, such as *Psacalium descompositum*, from which cacalol acetate has been isolated. This compound is responsible for the anti-inflammatory effect of this plant. However, such mechanism has not been explored.
The aim of this investigation was to establish whether the anti-inflammatory activity of cacalol acetate involves the inhibition of the NF-κB model *In vitro*. RAW 264.7 macrophages were cultured, pretreated with cacalol acetate and then stimulated with LPS. Nuclear and cytoplasmic proteins were isolated. Proteins of the NF-κB pathway (p65, IκBα, Ikkα) were measured by Western blot, as well as the relative expression of TNF-α, IL-6, IL-1β.
A decrease in the expression and release to the medium of TNF-α, IL-6 and IL-1β was observed. In Western blot, no changes were observed in IκBα in the groups that had been pretreated with cacalol acetate and then stimulated with LPS. In the groups that had been pretreated with the compound and then stimulated with LPS, p65 phosphorylation subunit in the nucleus decreased. These results suggest that the action of cacalol acetate could inhibit IκBα, which prevents the translocation of NF-κB to the nucleus. These effects may be associated with a decrease in the release of cytokines. It is important to continue studying the factors involved in the regulation of said inhibition, as well as of other transcription factors involved in the anti-inflammatory action of cacalol acetate.

Comparison of cytochrome P450 expression in mice under different housing conditions, and parasitosis

Q.FB. Mouret Hernández Circe Martha Alicia, Dr. Rafael Camacho Carranza, Dr. Víctor Daniel Garzón Cortés, Dr. Jesús Javier Espinosa Aguirre
Depto. de Medicina Genómica y Toxicología Ambiental, Instituto de Investigaciones Biomédicas, UNAM, Ciudad Universitaria, Deleg. Coyoacán, C.P. 04510, México, D.F., México. Tel: 562-29214, email: jjea99@gmail.com

Introduction: The environment is a factor that can generate changes in the composition of the microbiota, and this, in turn may lead to the modulation Cyp450 transcription, through nuclear receptors.

Rodents for research purposes are usually housed in various types of facilities, and it is even recurrent that they are parasitized with *Trichomonas muris*; factors that can have a substantial impact on the composition of the microbiota, and therefore on the modulation of P450 genes transcription. Therefore, the present study aimed to characterize changes in the expression of cytochromes P450 in mice housed in different types of animal facilities.

Methods: Male CD1 mice were divided into 3 groups of ten animals each: 1. Animal facility standard barrier, 2. Conventional animal facility, 3. Infected with *T. muris* (in conventional animal facility). Animals were kept at constant conditions with light-dark cycles of 12 h, fed ad libitum. The experimental protocol included the microbiological analysis of faeces. Infection with *T. muris* was achieved when infected females coexisted with male mice for 2 weeks, after this time, infection with *T. muris* was confirmed by microscopic analysis of the cecum. All the experimental groups were sacrificed at 12 weeks of age. The liver and intestine were collected and processed for quantification of the mRNA of Cyp1a2, Cyp2c66, Cyp3a11 and protein Cyp3a.

Results: Microbiological counts were not statistically different between groups. However, higher expression of Cyp1a2 and Cyp3a11 mRNA was observed in the animals housed in conventional animal facility.

Conclusion: Animals under conditions of animal facility standard barrier present a lower Cyp1a2 and Cyp3a11 mRNA expression.

Determination of the anticancer activity of the ethanolic extract *of Equisetum arvense*

Jesús Antonio Hernández López[1], Venancio Vanoye Eligio[1], Jorge Ariel Torres Castillo[1], Mónica Azucena Ramírez Cabrera[2], Eder Ubaldo Arredondo Espinoza[2], Fabián Eliseo Olazarán Santibáñez[1].
[1] Instituto de Ecología Aplicada, Universidad Autónoma de Tamaulipas.
[2] Facultad de Ciencias Químicas, Universidad Autónoma de Nuevo León.
Cd Victoria, México. C.P. 87019, Tel. (834) 31818-00 Ext 1612, feolazaran@uat.edu.mx

Abstract

Horsetail (*Equisetum arvense*, Equisetaceae) is a shrub used in traditional medicine for several purposes, it contains alkaloids, carbohydrates, proteins and amino acids, phytosterols, saponins, sterols, ascorbic acid, silicic acid, phenols like tannins, flavonoids, triterpenoids, volatile oils and many other biological active constituents. The pharmacological studies showed that it possesses antioxidant, anticancer, antimicrobial, anti-inflammatory, antidiabetic, diuretic, and other effects. Hence, the present study was conducted to investigate the anticancer effect of the ethanolic extract of *E. arvense*. The antiproliferative effect of the ethanolic extract was tested on SIHA cells. Compared to untreated cells, the plant extract showed an important cytotoxic effect on the cervix cancer cell line.

Effect of 1,2-Dimethylhydrazine in the kidney and heart of male Wistar rats.

Luis Gerardo Ortega-Pérez, Patricia Ríos-Chávez, Yazmin Carreón-Abud
[1]Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo.
Cd. Universitaria, C.P 58030, Morelia, Michoacán, México.
herpebio@hotmail.com

Oxidative stress has an important role in the incidence of kidney and heart diseases. 1,2-dimethylhydrazine (DMH) is a toxic compound, which was reported as a specific colon procarcinogen, DMH is metabolized until diazonium ion which elicits an oxidative stress and produced inflammation and tumor promotion, however there is not study about the effect of this compound in other organs. *Callistemon citrinus* is a plant that produced compounds that could have pharmacological applications by its antioxidant capacity. The aim of this study was to determine if 1,2- dimethylhydrazine could causes damage in the antioxidant system in the kidney and heart of Wistar rats and to see the protective effect of *C. citrinus* extract in these organs. 24 male albino Wistar rats were randomly divided into four groups (n = 6). Group I was control; Group II, III and IV received 5 subcutaneous injections of 1,2-dimethylhydrazine (65 mg/kg) daily for 3 weeks; Group III and IV received extracts of *C. citrinus* leaves and flower respectively (250 mg / kg) daily for 22 consecutive weeks.

The activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase and quinone reductase showed a decrease in DMH treated rats, whereas in the rats administered with leaf extracts showed an increase in the activity of these enzymes in relation to the control, whereas the treatment group with the flower extract showed a decrease in all the enzymes determined in the kidney, our results demonstrated beneficial effects of leaf extract in the kidney and heart.

Key words: oxidative stress, free radicals, 1,2-dimethylhydrazine, antioxidants

Toxic activity of secretions from parotid glands of *Rhinella marina*

Andrea Peñaloza Cabrera[1], Rita Restano Cassulini[2], Luis Caspeta Guadarrama[2] and Lidia González Morales[1].
[1]Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos. [2]Instituto de Biotecnología, Universidad Nacional Autónoma de México. Av. Universidad No. 1001, Col Chamilpa, Cuernavaca, Morelos, México. C.P. 62209. Tel. 777 3297057. Email: lidia.gonzalez@uaem.mx.

Secretions from anuran skin have developed interest in the search for novel bioactive components. Parotid glands secretions from *Rhinella marina*, has been subject of investigations by the presence of molecules with many biological activities. However, the toxic effects produced by these secretions on mammals, arthropods or ionic channels are scarce. In this work we evaluated the toxic effects of the total venom from parotid glands of *Rhinella marina* from Colima state on mammals, arthropods and ionic channels. The toxicity of the secretions was assayed in mice (*Mus musculus*) and crickets (*Acheta domesticus)*. Severe symptoms such as paralysis, convulsions and death were observed in mice. While in crickets only partial paralysis was observed, A LD_{50} of 355 mg/kg for mammals was also determined. The damaged produced in ionic channels, was evaluated via electrophysiological assays on several sub-types of voltage-gated Na_+ channels. Secretions block these channels in approximately 50% applying 1 mg/ml dose, while at concentrations of 10 mg/ml block almost completely. This is the first report of the electrophysiological effects produced by secretions from the parotid glands of the Mexican toad *Rhinella marina*.

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¨Glutamate Receptor Modulation by Chronic Arsenic Exposure in drinking water¨

Wendy Leslie González Alfonso [1], Janikua Florita Nelson Mora, Pavel Petrosyan, María Eugenia Gonsebatt Bonaparte [2]
Departamento de Medicina Genómica y Toxicología Ambiental, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México
Apartado postal 70228, Mexico City 04510, Mexico
Tel. +52 (55) 56229178
[1] wlglez93@gmail.com, [2] mgonsebatt@gmail.com

Inorganic arsenic (iAs) constitutes an obicuous metalloid, epidemiologically considered a natural pollutant in countries like Argentina, Chile, India, Mexico and United States of America. Chronic exposure to iAs in drinking water during gestation and in early stages of life, has been associated important neurological affectation, such as deficit in memory and learning.

Inside the cell, iAs and its methylated species can alter signaling pathway, redox balance, DNA integrity and genetic expression. The depletion of GSH, during the arsenic metabolism, is considered one of the most important mechanism of redox imbalance and neurological damage.

Alterations in the glutamatergic synapsis is one of the most described neurotoxic effect of iAs in animal models. A decrease in the number of presynaptic vesicles, changes in glutamate receptor and postsynaptic density protein expression, as well as signaling pathways impairment have been observed. We have shown altered glutamate disposition probably caused by the decreased expression of GLT1 and GLAST glutamate transporters and an upregulation of xCT in mice exposed to iAs during gestation. This changes in the hippocampus were associated with a lower expression of NR2B NMDA receptor subunit.

In this work, we study the modulation of AMPA receptor expression in the hippocampal region. To reach this goal, CD1 mice were chronically and gestationally exposed to 20ppm of iAs in drinking water. The levels of GluA1 protein and mRNA expression as well as markers of GluA1 vesicular traffic will be determined in 3 month old mice hippocampus.

Chemopreventive effect of Callistemon *citrinus* on colorectal cancer in male Wistar rats.

Patricia Ríos-Chávez[1], Alejandro López Mejía[1], Oliver Rafid Magaña-Rodriguez[1], Daniel Godínez Hernández[2], Blanca Nateras Marin[2].

[1]Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo. Cd. Universitaria, C.P 58030, prchavez17@gmail.com. Morelia, Michoacán, México. [2]Instituto Químico-Biológicas Universidad Michoacana de San Nicolás de Hidalgo, Edificio B-2, Cd Universitaria, Morelia, Michoacán., México

Colorectal cancer is an important public health issue because is one of the most frequent cancers and have a high mortality rate. The search of bioactive compounds from plants with anticancer and chemopreventive properties have a prominent place in research against this disease The aim of this study was to determine the protective effect of the extracts of *C. citrinus* of leaves and flowers in a model of colorrectal cancer induced in rats. Four experimental groups (n = 8) were established: one control, and three that were administered with 5 doses (65 mg/kg) of DMH. Two of these groups were administered with daily oral doses (250 mg/kg) of leaves and flowers extracts (respectively) during the all the experiment, which lasted 22 weeks. The animals were sacrificed and the presence of tumors and aberrant cryptic foci (ACF) were determined. The leaf extract had an effect in reducing the number of animals with tumors by 33% against 100% of the group only treated with DMH. The number of ACF was also reduced in the group treated with the leaves extract (15 against 36) with respect to the DMH group. In contrast, the flower extract had a negative effect and only one individual survived that treatment. The activity of three antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) and two of phase II (quinone reductase and glutathione S-transferase) in liver, proximal, middle and distal colon were also evaluated. The extracts of *C. citrinus* promoted the enzymatic activity, being equal to or higher to the control group. In contrast, in the DMH treated group, the enzymatic activity decreased in most of the tissues analyzed. Additionally, the content of glutathione (GSH), was evaluated as a marker of oxidative stress damage. Again, the GSH content decreased significantly in the negative control group and increased or remained normal in the groups treated with the extracts respect to the control. The results obtained show that *C. citrinus* has a protective effect via enzymatic induction and reduce oxidative stress damage in colorectal cancer.

Keywords: *Callistemon citrinus*, chemoprevention, colorrectal cancer, enzymatic induction, antioxidant activity.

Effects of pharmacological concentrations of the vitamin biotin in the kidney

(1)Leticia Riverón-Negrete, (2)Karina Pasten-Hidalgo, (1) Gustavo Canul-Medina, (3)Carlos Cedillo-Peláez, (1)Gloria Sicilia-Argumedo, (1) Fernández Mejía Cristina.
(1) Unidad de Genética de la Nutrición, UNAM/INP.
(2) Cátedra CONACyT, Unidad de Genética de la Nutrición, UNAM/INP.
(3) Laboratorio de Inmunología Experimental, INP
Avenida del Imán 1, 4th Floor, Colonia Insurgentes Cuicuilco, 04530 México City, México. Email: cristinafernandez55@gmail.com

Biotin is a water-soluble vitamin; at pharmacological concentrations about 30-650 times its recommended diary requirements have favorable effects on triglycerides and glucose homeostasis. These effects suggest that biotin could be used for the treatment of metabolic syndrome and diabetes. However, there is scarce information about whether biotin has toxic effects at concentrations that produce their beneficial effects on glucose and lipids homeostasis. In previous studies we found that, in an animal model in which biotin supplementation produced favorable metabolic effects it also produced morphological changes in the pancreatic islets and in the liver. In the present study we analyzed the effects of biotin supplementation on kidney histomorphology and diferent serum kidney damage markers. Two groups of male mice were fed during 8 weeks a control diet containing 1.76 mg biotin/ food kg or a biotin supplemented diet 97.7 mg biotin/ food kg (Harlan Teklad, Madison WI, USA). After 8 weeks of feeding, mice were deprived of food for 12 h, anesthetized with Sevorane, and the blood and kidneys were extracted. Finally, the mice were killed via cervical dislocation. The concentration of albumin, urea and uric acid were determined as markers of renal function. The histological analysis was determined in kidney sections stained with hematoxylin-eosin. All data are presented as the mean±S.E.M. Statistical analyses were performed using the Statview statistical analysis program GraphPad Prism

6.0 software Berkeley, CA, USA). P values less than 0.05 were considered statistically significant. The results showed that versus the control mice, biotin-supplemented mice presented 42.3% of capsule loss space ($p<0.05$). No differences were observed in serum albumin, creatinine and urea concentrations. The results indicated that biotin supplementation in the diet produced loss of glomerular capsule space; however the histological changes were not reflected in markers of kidney damage function. In conclusion, these results indicate that biotin toxicity studies need to be addressed with different tools in the view of the fact that pharmacological concentrations of biotin affect tissue morphology without affecting classical functional markers.

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Pharmacological effect of biotin on the development of adipose tissue

Gustavo Rojas-Olave[1], Wilma Tixi-Verdugo[1], Ericka Moreno-Mendez [1], Gloria Sicilia-Argumedo[1], Cristina Fernández-Mejía[1, 2], [1]Unidad de Genética de la Nutrición, [1]Instituto de Investigaciones Biomédicas, [1]Universidad Nacional Autónoma de México, Instituto Nacional de Pediatría. Avenida del Imán 1, cuarto piso, Colonia Insurgentes Cuicuilco, 04530 Ciudad de México. Tel. 56226420. 2crisfern@biomedicas.unam.mx

Introduction. Biotin is a water-soluble vitamin that acts as carboxylase prosthetic group; in addition, at pharmacological doses modify several functions, among them lipid metabolism. In previous studies we demonstrated that eight weeks of a biotin-supplemented diet after weaning decreased the expression of genes of adipose tissue development, such as like transcription factor SREBP-1c and pro-adipogenic factor PPARγ, which suggest that biotin might modify the adipose tissue development. In the present study we investigated the effects of biotin-supplementation during rodent adipose tissue development, gestation and lactation, on offspring adipose tissue morphology and gene expression.

Methods. Adult female BALBc/ANN Hsd mice were fed a biotin control or a biotin supplemented diet (1.76 mg and 97.7 mg of biotin/kg diet, respectively) during pregnancy and lactation. At d21 of postnatal life (weaning day) body weight was determined, and different adipose tissue depots were removed from female offspring. Tissue morphology was performed in sections stained with hematoxilin-eosin and analyzed with Image J 1.4 software. Relative abundance of mRNA was analyzed by Q-RT-PCR.

Results. The data showed that, compared to the control group, the offspring resulted from mothers that received biotin-supplementation during pregnancy and lactation decreased weight and size of perigonadal, inguinal and interescapular adipose tissue depots. The subcutaneous inguinal depot analysis found that biotin-supplemented diet decreased white adipocyte area, increased the mRNA abundance of adipogenic gen Pref-1 and decreased mRNA of leptin gen; taken together these data suggest that biotin supplementation diminished subcutaneous inguinal white adipocytes adipogénesis. In contrast in the perigonadal adipose tissue, Pref-1 expression was not modified but we found increased mRNA abundance of beige adipocytes markers genes: UCP1, CIDEA and Cox 8b, results that suggest that biotin-supplementation increased beige adipocytes in the perigonadal adipose tissue depot.

Conclusions. Biotin supplementation during gestation and lactation diminishes adipose tissue content and modifies adipose tissue morphology and gene expression. This effect differs between adipose tissue depots. The present investigation shows for the first time that biotin-supplementation modifies adipose tissue development.

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Bio-guided fractionation of an extract of *Cucurbita ficifolia* Bouche, and effect of some fractions on insulin secretion and GLUT-4 expression.

Wendoline Rosiles Alanis[*] (wendie04@hotmail.com), Julio Almanza Perez[1], Rebeca García Macedo[1], Alejandro Zamilpa Álvarez[2], Beatriz Mora Ramiro[*], Francisco Javier Alarcón-Aguilar[1], Rubén Román Ramos[1].

[*]Posgrado en Biología Experimental. Universidad Autónoma Metropolitana – Iztapalapa. (wendie04@hotmail.com)
[1]Lab. Farmacología, Depto. Ciencias de la Salud, Universidad Autónoma Metropolitana-Iztapalapa. San Rafael Atlixco 186, Vicentina, Iztapalapa, C.P. 09340, Ciudad de México.
[2]Centro de Investigación Biomédica del Sur (IMSS) Argentina No. 1 Xochitepec Morelos.
3Unidad de Investigación Bioquímica, Centro Médico Nacional, Siglo XX1, IMSS, Ciudad de México.

Keywords: Diabetes Mellitus, Cucurbita ficifolia

Diabetes Mellitus comprises a group of metabolic diseases, characterized by a sustained state of hyperglycemia that results from defects in the secretion of insulin by pancreatic cells or action of it in its target tissues, such as muscle and adipose tissue, which play a central role in the regulation of glucose metabolism throughout the body[1]. Chronical hyperglucemia, which is present in Diabetes, is highly associated with damage to tissues and organs including kidneys, nerves, eyes, heart and vascular system. Although there are different treatments for a multifactorial and complex disease such as Diabetes, the population continues to resort to traditional medicine, which is why it is important to carry out phytochemical studies of the species that are used. *Cucurbita ficifolia* is an example of species commonly used to treat diabetes, and in previous studies it has been demonstrated to have a wide range of effects, such as hypoglycemic, antioxidant and anti-inflammatory.

The aim of this work is to carry out a bio-effect directed fractionation of an extract of *Cucurbita ficifolia*, in order to obtain less complex fractions and possibly isolate compounds that are responsible of the antidiabetic effects.
Three different extracts of Cucurbita ficifolia were elaborated; ethyl acetate extract, acetone and aqueous extract, and evaluated in an *In vivo* model, in order to choose the extract with greater activity and make subsequent primary and secondary fractionation. Both the fractions and the sub-fractions obtained from this process were evaluated *In vitro* in pancreatic cells for insulin secretion and in myocytes for expression of glucose transporter GLUT-4.

The aqueous extract showed an hypoglycemic effect in the acute *In vivo* test, however, the fraction resulting from a split in ethyl acetate of the same extract, was found to have anti-hyperglycaemic effect in glucose tolerance tests, in the same way as some of its primary sub-fractions.
Most of the secondary sub-fractions showed an increase in insulin secretion, while regarding GLUT-4 expression, only two of the primary sub-fractions and some of the secondary sub-fractions showed a significative rise. These results suggest that the anti-hyperglycaemic and hypoglycaemic effects, might be given through different mechanisms and involve different tissues or cells.

Participation of HIF-1 in the regulation of pharmacologically induced autophagy in breast and colon tumor cells

Rebeca Salgado-García[1], Izamary Delgado-Waldo[1], Nadia Jacobo-Herrera[2], Eduardo López-Urrutia[3] and Carlos Pérez-Plasencia[1,3].
Instituto Nacional de Cancerología, Av. San Fernando No. 22, Col. Sección XVI Delegación Tlalpan, C.P. 14080 México, D.F[1].
Instituto Nacional de Ciencias Médicas y de Nutrición Salvador Zubirán, Unidad de Bioquimica, México, Distrito Federal[2].
Universidad Nacional Autónoma de México UNAM, FES-Iztacala, UBIMED; Laboratorio de Genómica Funcional. Tlalnepantla, Estado de México[3]
Correspondence author: Av. San Fernando No. 22, Col. Sección XVI Delegación Tlalpan, C.P. 14080 México, D.F. carlos.pplas@gmail.com

Colorectal cancer (CRC) and breast cancer (CaMa) are a worldwide health problem with high incidence and mortality rates in both sexes. Previous research has shown that the initiation and progression of CRC and CaMa may be associated with deregulation of essential pathways (such as PI3K / AKT / mTOR), which is of paramount importance since it is responsible for the synthesis of the HIF-1 transcription involved in the regulation of various biological processes such as apoptosis and autophagy. Autophagy is an evolutionarily conserved multi-step process where the cell degrades its long-lived proteins and damaged organelles. Our research group was interested in studying the induction of this process in cell line HCT116 and MDA-231 by combining three drugs: Metformin and Sodium Oxamate, inhibitors of mTOR and glycolysis respectively, in synergy with Doxorubicin which is a conventional chemotherapeutic. A decrease in the detection of proteins involved in the PI3K pathway and an increase in the detection of autophagy inducing proteins were demonstrated by Western blotting in cells treated with the three drugs in combination. Subsequently, the expression level of miR-106a involved in autophagy was evaluated, showing a modification of the expression in the cell lines exposed to the treatments at the different times. Luciferase assays showed that ULK1, the main autophagy inducer, is directly regulated by miR-106a. In conclusion, the present work will provide detailed information on the modulation processes of the HIF-1 signaling network and its regulation on the autophagy process, which will allow us to propose better and effective therapeutic strategies against CRC and CaMa.

Key words. Colorectal cancer, Breast cancer, Autophagy, HIF-1, metformin, sodium oxamate, and doxorubicin.

Curcumin effective dose against oxidative stress linked with hepatic insulin resistance development by exposure to cadmium.

Victor Enrique Sarmiento-Ortega, Eduardo Brambila, Diana Moroni, Alfonso Diaz, Samuel Treviño.
Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla.
Avenida San Claudio SN, CP.72592
Tel. 2295500 Ext. 7370
samuel_trevino@hotmail.com

Cadmium (Cd) is considered the fifth environmental pollutant in the world. Liver and kidneys are the main target tissues of Cd toxicity. Cd exposition is principal one of the concerns for public health since agricultural and industrial activities favor exposure to metal by means of air, soil, and water. Our research group has already shown that Cd acts as a metabolic disruptor of the glucose and lipid homeostasis, as well as produces insulin resistance in Wistar rats after an exposition of 32 ppm of Cd in drinking water (NOAEL dose) for 25 - 30 days. While the mechanism by which the metal induces insulin resistance is unknown, the oxidative stress could be the link. Therefore, the present study focuses on the role of oxidative stress as an underlying mechanism in the development of insulin resistance induced by Cd. In animals exposed for 25 days to 32 ppm of Cd in drinking water at *libitum*, which presented insulin resistance (HOMA-IR 125% and Liver Insulin Resistance Index (101%), dysglycemia (24%) and dyslipidemia associated to triglyceridemia (28%), LDL-chol increase (145%) and HDL-chol diminish (28%) in relation to animals non-exposed. Additionally, rats-exposed accumulate Cd (14242%) in the liver and increase ROS (405%), NO_2^- (105%), MDA (97%) and 4-HDA (104%). Curcumin, a potent antioxidant was administered oral via, in doses of 50 mg, 125 mg, 250 mg, 500 mg and 750 mg/kg, daily for 5 days. In order to know the effective dose, animals were sacrificed and evaluated for insulin resistance, dysglycemia, and dyslipidemia, as well as transaminases, creatinine and urea such as liver and kidney-toxicity biomarkers. At hepatic level were reevaluated levels of cadmium, ROS, NO2-, MDA, and 4-HDA. Effective dose corresponded to 250 mg/kg, which showed a notable improvement on glycemia (8%), triglycerides (39%), LDL-chol (39%) and HDL-chol (48%), as well as on cadmium toxicity in liver and kidney, diminishing transaminase (ALAT 12%), urea 12% and creatinine 12%, respectively. Likewise, HOMA-IR and LIRI indexes improved at a level close to control, which strongly correlated with the diminishing of hepatic levels of Cd (13%), ROS (53%) and NO_2^- (42%). In conclusion, our results provide evidence of hepatic insulin resistance developed after an exposure to NOAEL cadmium dose. Also, the use of curcumin as an antioxidant and chelator (250 mg/kg/5 days) improved complications of cadmium metabolic toxicity. Finally, our results strongly suggest that cadmium act as a metabolic disruptor that involved oxidative stress generation in hepatic insulin resistance development.

Extraction and identification of a cardiotoxic fraction of the venom of *Condylactis gigantea* from Mexican Caribbean

María Vanegas Reza1 (vanerma05@gmail.com), Ana Laura Sánchez Sandoval2 (alss_7@yahoo.com.mx), Roberto Alejandro Arreguín Espinosa de los Monteros1 (arrespin@gmail.com), Juan Carlos Gómora Martínez2 (jgomora@ifc.unam.mx), Judith Sánchez Rodríguez3 (judithsa@cmarl.unam.mx)**.**
1 Instituto de Química, Dpto. Química de Biomacromaléculas, UNAM, Circuito Exterior S/N., Ciudad Universitaria, Coyoacán, Cd. Mex, México CP. 04510. Tel. (01-55) 5622-4516 y 4468.
2 Instituto de Fisiología Celular, Departamento de Neuropatología Molecular. UNAM
3 Laboratorio de Toxinología de Cnidarios, Unidad Académica de Sistemas Arrecifales de Puerto Morelos, UNAM. Puerto Morelos, Q. Roo, México.

Abstract.
The anemone *Condylactis gigantea* is a benthic organism normally associated with the hard substrate of reef environments. In Mexico, its distribution is restricted to the Caribbean Sea and the northern Yucatan Peninsula. As a member of the Cnidaria phylum, it is characterized by having cnidoblasts, which may have nematocysts, which harbor the venom of these animals; this is a complex set of toxins with different targets, and therefore, represent a source of compounds with potential for biotechnological purposes.
Currently there are numerous reports on neurotoxins from anemones; among which various peptide compounds with different action targets are described. Among which, the most studied are those that act on sodium channels dependent on voltage (Nav); being the first reported with toxins of *Anemonia sulcata* and *Condylactis gigantea* in 1989. Since those reports, more than 50 anemone toxins have been isolated, and others produced by recombinant DNA techniques1; on which has been discovered that most act by slowing the inactivation of the channel during the depolarization process.[2]
In this research work, a fraction of the *C. gigantea* venom, with reversible inactivation effect on voltage-dependent sodium channels 1.7 (Nav 1.7) was identified, by *in vitro* Patch-Clamp assays in HEK293 cells. These channels are representative of cardiomyocytes, and alterations in their functioning cause conditions such as Brugada syndrome and long QT, in addition they have been related to the proliferation of cancer cells and the initiation of metastatic processes, when they appear in tissues where normally its expression is null or limited. Therefore, it is important to find and describe substances that can help in the treatment of these diseases

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Diversification of tRNA genes through duplication in *Cucurbita*

Xitlali Aguirre Dugua[1],*, Leslie M. Paredes Torres[2], Helena S. Hernández Rosales[2], Paulina Hernández[2], Karen Ruiz Mondragón[2], Gabriela Castellanos Morales[3], Guillermo Sánchez de la Vega[2], Josué Barrera Redondo[2], Enrique Scheinvar Gottdiener[2], Erika Aguirre Planter[2], Luis E. Eguiarte Fruns[2], Rafael Lira Saade[1], Salvador Montes Hernández[4]
[1]Unidad de Biotecnología y Prototipos, Facultad de Estudios Superiores Iztacala, UNAM. Av. De Los Barrios 1, Los Reyes Iztacala, Tlalnepantla Estado de México.
*xitla.aguirre@gmail.com
[2]Departamento de Ecología Evolutiva, Instituto de Ecología, UNAM.
[3]El Colegio de la Frontera Sur Unidad Villahermosa.
[4]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias Campo Experimental Bajío.

Different genes of chloroplast origin have previously been identified in the mitochondrion genome of *Cucurbita pepo* (pumpkin; Cucurbitaceae). These include the tRNA encoding *trnT* and *trnL*. The *trnT-trnL* intergenic region found in the chloroplast genome has been widely used as a molecular marker for phylogenetic studies at the genus and family levels, and specific mutations have therefore been identified within particular *Cucurbita* lineages. However, little is known on the phylogenetic distribution of the mitochondrial copy and its molecular charac teristics. In this work we compare the mitochondrial and chloroplast copies of the *trnT-trnL* region in ten species of *Cucurbita*, including cultivated (three species) and wild representatives (six taxa), at the sequence and structural levels. The mitochondrial and chloroplast copies were PCRamplified (>900 and >700 bp, respectively) and Sanger-sequenced. The mitochondrial copy was successfully amplified in six taxa (all four cultivated and two wild taxa) belonging to the *Cucurbita* mesophytic clade, whereas no product was obtained in the five xerophytic taxa. Gene diversity was compared between the two groups of copies with the nucleotide diversity index _, whereas sites under selection were identified using Tajima's *D*. The secondary structure of the 50 bp tRNA sequence was predicted using the minimum free energy model.
Our results suggest that the transfer of the analyzed region from the chloroplast to the mitochondrion genome occurred in the common ancestor of the mesophytic clade. The high substitution rate observed in the *Cucurbita* mitochondrion has generated a noticeable divergence between the two copies, which is directly related to the functional diversification of the encoded tRNAs.

Real-Time Monitoring of *Nicotiana benthamiana* Volatile Response to Microbial Scents by Low-Temperature Plasma Mass Spectrometry

Raúl Alcalde Vázquez[1,2], Abigail Moreno Pedraza[1], Laila Pamela Partida Martínez[2] and Robert Winkler[1]
Centro de Investigación y de Estudios Avanzados del IPN, Unidad Irapuato.
Departamento de Biotecnología y Bioquímica[1] y Departamento de Ingeniería Genética[2].
Km 9.6 Libramiento Norte, carretera Irapuato-León. C.P. 36824. raul.alcalde@cinvestav.mx

All living organisms must identify cues from their environment in order to adapt and survive. In most biological systems, chemicals serve as important environmental signals. Among these chemicals, volatile organic compounds (VOCs) represent a large and diverse group of molecules that plants use for both intra and inter-organismal communication. Although plant volatiles have been studied for decades, it remains unclear in what ways plants adjust their volatile profiles in response to VOCs of nearby microorganisms (mVOCs). Here we investigate how plant volatiles change, both in composition and across time, when plants had been exposed to microbial scents.

Low-Temperature Plasma Mass Spectrometry (LTP-MS) is an ambient ionization technique that allows the measurement of molecules in real-time[a]. In this work we use LTP-MS to monitor the volatile response of *Nicotiana benthamiana* after exposure to characterized microbial scents[b]. As a first step, we selected the microbial VOC 2-phenyl ethyl alcohol as elicitor. Plant-emitted volatiles were measured in a continuous mode for 45 min, of which the first 5 min represented the plant basal emission, the next 20 min corresponded to the response during induction as the elicitor was added into the system, and the last 20 min corresponded to the response after induction once the elicitor was removed.

We observed a dynamic volatile response in which different mass ions were detected and monitored during time. This experiment served as the proof of concept for the on-line measurement of plant volatiles in response to specific elicitors. Future studies will focus on expanding our understanding of microbe-plant communication, as well as improving this technology to expand investigations of VOC-mediated interactions.

Key words: Plant volatile organic compounds, microbial scents, plant-microbial interactions, Low-Temperature Plasma Mass Spectrometry.

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Subcellular Localization of long non-coding RNA Using Modified Two-Component System in Plants

Jaime Aportela Cortez1, Coral Martínez Martínez2, Alejandra A. Covarrubias Robles2, Catalina Arenas Huertero1*.
Instituto de Biotecnología, Universidad Nacional Autónoma de México2, Facultad de Ciencias, Universidad Autónoma de San Luis Potosí1. Diagonal Salvador Nava Mtz s/n, Zona Universitaria, San Luis Potosí, SLP. México. CP 78290.
catalina.arenas@uaslp.mx1*

During the last years the transcriptome and RNA-seq analyses have shown the relevance of RNA molecules and their function during the cell life cycle. Also, recent information shows that the functions of these RNA molecules are more diverse and tightly controlled than were previously thought. All of these studies suggest that intergenic and non-coding regions are transcribed genome-wide and that many of these transcripts correspond to long non-coding RNAs (lncRNAs). It has been reported that the transcript levels for many lncRNAs are regulated in tissue/organ-specific and/or stress-dependent manner. In order to investigate the role of some of the long lncRNAs and their possible molecular mechanisms in the cell, we pursue their subcellular localization, among other strategies. For this, we applied a new approach using the “Two-Component System” (Kinoshita et al 2017, submitted).

This approach implied the modification of the method developed by Schonberger et al. (2012) to be used in transient expression experiments. This technique requires: a) an RNA component plasmid containing a highly structured RNA from MS2 bacteriophage, and mCherry as the reporter gene for transformation; and b) a GFP protein component containing the MS2 RNA binding protein fused to a nuclear exportation sequence (NES). We used this method to determine the subcellular localization of three Arabidopsis thaliana lncRNA candidates, with an unknown function. Our results showed that two of three lncRNAs have a nuclear localization, independently of NES. These data suggest that lncRNAs could participate in nuclear regulatory process of gene expression.

Cold tolerance and RNA binding activity of the AtGRDP2 protein in *Arabidopsis thaliana*

Saraí Castro Bustos, María Azucena Ortega Amaro, Israel Maruri López, Mitzi Xereni Cortés Amaro, Alicia Becerra Flora and Juan Francisco Jiménez Bremont.
División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica (IPICYT) AC, Camino a la Presa de San José 2055, C.P. 78216. San Luis Potosí, SLP, México. Email: jbremont@ipicyt.edu.mx, (+52) 444-8-342000.

Plant genomes encode a widely variety of RNA binding proteins (RBPs) that display multiple roles controlling post-transcriptional RNA metabolism such as mRNA export, pre-mRNA splicing, localization and chaperone activity. Additionally, it has been reported that RBPs are involved in growth, development and stress response, such as cold and freezing. Arabidopsis *AtGRDP2* gene encodes a short glycine-rich domain protein containing a DUF1399 domain and a putative RNA recognition motif (RRM). Under low temperatures assays 35S::AtGRDP2 over-expression lines showed a higher tolerance in comparison to WT plants, while the *Atgrdp2* mutants showed the opposite phenotype. Although *AtGRDP2* gene has been characterized, less information about the protein such as RNA-binding activity and localization has been demonstrated. Here, we report *in vivo* assay of anti-transcription termination test in the *E. coli* RL211 strain revealing the possible function of AtGRDP2 protein as RNA chaperone in the heterologous system. Transient expression AtGRDP2-GFP in tobacco showed the subcellular localization of AtGRDP2 protein in cytosol and chloroplasts. These data are key to continue with the functional characterization of AtGRDP2, and its involvement in the plant response to abiotic stress.

Evaluation of gene expression and translational status in the silencing line amiR: *ADC-L2* of *Arabidopsis thaliana* using polysome profiles and detection of transcripts

Montserrat Chacón-Flores[1], Ana Isabel Chávez-Martínez[1], Tzvetanka D. Dinkova, Alicia Becerra Flora[1] and Juan Francisco Jiménez-Bremont[1].
[1]División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica (IPICYT) AC, Camino a la Presa de San José 2055, C.P. 78216. San Luis Potosí, SLP, México. (+52) 444-8-342000. jbremont@ipicyt.edu.mx
[2]Departamento de Bioquímica, Facultad de Química, Universidad Nacional Autónoma de México, 04510, México, D. F., México

Polyamines (PAs), are low molecular weight polycationic aliphatic amines, highly distributed in all forms of life. The main PAs are putrescine (Put), spermidine (Spd) and spermine (Spm). Particularly, in plants these amines are important in several growth and development processes. Also, due to the polycationic nature, PAs are able to have electrostatic or covalent binding to various anionic macromolecules. In this sense, at cellular level, PAs can be implicated in modulation of gene expression, maintaining mRNA stability and translation regulation. However, even though is believed that, specific and global translation events are orchestrated by PAs, their specific functions in vivo are poorly understood. Between the evidence of PAs participation in translation process, it is known that one property that is being researched is the hypusine of the cofactor eIF-5A on protein synthesis, indicating that eIF5A works as a sensor and effector for polyamine control of translation. Hypusine is a post-translational modification in a lysine of eIF5A, which are formed with contribution from the Spd. Recently, we generated an *A. thaliana ADC* silenced line (amiR:*ADC*-L2) that succeeded to have a drastic reduction in PAs levels. This line exhibits defects in growth and development. In order to analyze at translational level the effect of reduction of PAs, we evaluate the differences observed in ribosomal profiles between the ecotype WS-0 and amiR:*ADC*-L2 line, as well as the differences with exogenous application of Put at 10µM. Also, we analyze some specific transcripts such as PAs transporters with the aim of elucidating the mechanism that cell performs when the PA content is re-established exogenously.

Generation of scientific and technological strategies with a multidisciplinary and interinstitutional approach to face the threat represented by exotic ambrosia beetles to the agricultural and forestry sectors of Mexico[£]

José A. Guerrero-Analco1, Enrique Ibarra-Laclette1, Frédérique Reverchon1, Juan L. Monribot-Villanueva1, Eric E. Hernández-Domínguez1, Claudia A. Pérez-Torres1, José L. Olivares-Romero1, Eliel Ruiz-May1, Andrés Lira-Noriega1, Emanuel Villafán1, Israel Bonilla-Landa1, Randy Ortiz-Castro1, Araceli Lamelas-Cabello1, Nadia L. Caram-Salas1, Ofelia Ferrera Rodríguez1, Ana L. Kiel-Martínez1, José B. Rodríguez-Haas1, José M. Elizalde-Contreras1, Martín Mata-Rosas1, Alma R. Altúzar-Molina1, Luis A. Ibarra-Juárez1, Felipe Barrera-Méndez1, Mónica Ramírez-Vázquez1, Fernando Rojano-Aguilar1, Daniel González-Tockman1, Larissa Guillen1, Aldo Segura-Cabrera1, Alexandro Alonso-Sánchez1, Lorena López-Sánchez1, Olinda E. Velázquez-López1, Greta H. Rosas-Saito1, Nicolaza Pariona-Mendoza1, Federico Escobar1, Hamilton Gómez de Oliveira1, Marco A. Castillo-Campohermoso2, Saúl Sánchez-Valdés2, Eduardo Ramírez-Vargas2, Florentino Soriano-Corral2, Adriana Espinoza-Martínez2, Carlos J.Espinoza-González2, Jorge Romero-García2, Ana M. Rodríguez-Hernández2, Antonio Serguei-Ledezma2, José H. Valenzuela-Solto2, Mona Kasem- Abbas2, Ramiro Guerrero-Santos2, Arxel de León Santillán2, Alejandro Alarcón3, Armando Equihua-Martínez3, Ronald Ferrera Cerrato3, Luis R. Herrera-Estrella4, Alfredo Herrera-Estrella4, Araceli Oropeza-Aburto4, Pedro Martínez4, Lenin Yong-Villalobos4, Claudio Ríos-Velasco5, Víctor M. Loyola Vargas6, Oscar A. Moreno Valenzuela6, Cecilia Hernández-Zepeda6, Gabriela Rosiles-González6, Marianne Luna-Rivero6, Yereni Minero-García6, Citlali Colín Chávez7, Fabiola Esquivel-Chávez7, Rodolfo Fulgencio-Negrete7, Alejandro De Las Peñas-Nava8, Lina Riego-Ruiz8, Irene Castaño Navarro8, Sergio Casas-Flores8, Francisco Paraguay-Delgado9, Rafael Espinoza Luna10, Gerardo R. Flores Colunga10, Juan M. Torres-Rojo11, David R. Heres del Valle11, Alejandro J. López-Feldman11, Arturo I. Martínez-Enríquez12, José A. López Buenfil13, Clemente García-Avila13, Andrés Quezada-Sálinas13, Magnolia Moreno-Velazquez13, José M. Cambrón Crisantos13, José G. Torres-Martínez13, Lervin Hernández-Ramos13, Nayeli Carrillo-Ortiz13, Ana K. Preuss-Angeles13, José A. Lozada-García14, Andrés Moya15, Daniel Carillo16, Thomas H. Atkinson17, Akif Eskalen18, Martín R. Aluja1*, Diana Sanchez-Rangel1*.
1Instituto de Ecología A.C. (INECOL), Xalapa, C.P. 91070, MX.; 2Centro de Investigación en Química Aplicada (CIQA), Saltillo, C.P. 25294, MX.; 3Colegio de Posgraduados (COLPOS), Texcoco, C.P. 56230, MX; 4Laboratorio Nacional de Genómica para la Biodiversidad (LANGEBIO), Irapuato, C.P. 36821, MX; 5Centro de Investigación en Alimentación y Desarrollo, A.C. (CIAD), Hermosillo, C.P. 83304, MX; 6Centro de Investigación Científica de Yucatán (CICY), Mérida, C.P. 97205, MX; 7Centro de Innovación y Desarrollo Agroalimentario de Michoacán (CIDAM), Morelia, C.P. 58341, MX; 8Instituto Potosino de Investigación Científica y Tecnológica A.C. (IPICYT), San Luis Potosí, C.P. 78216, MX; 9Centro de Investigación en Materiales Avanzados, S.C. (CIMAV), Ciudad Juárez, C.P. 32330,

MX; 10Centro de Investigación en Óptica (CIO), León, C.P. 37150, MX; 11Centro de Investigación y Docencia Económicas (CIDE), Ciudad de México, C.P. 01210, MX; 12Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-Saltillo), Ramos Arizpe, C.P. 25900, MX; 13Centro Nacional de Referencia Fitosanitaria (CNRF) – Dirección General de Sanidad Vegetal (DGSV)-Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria (SENASICA), Tecámac, C.P. 55740, MX; 14Universidad Veracruzana (UV), Xalapa, C.P. 91090, MX, 15Universitat de València, València, 46010, Spain; 16University of Florida, Homestead, 33031, USA; 17University of Texas at Austin (UT), Austin, 78712, USA; 18University of California-Riverside (UCR), Riverside, 92521, USA. £This research is being developed under the collaborative framework of the Scientific and Technological BioMimic® Cluster. Corresponding: diana.sanchez@inecol.mx, martin.aluja@inecol.mx, Tel. (228)-842-1800.

Ambrosia beetles (AB) *Xyleborus glabratus* and *Euwallacea* sp. (Polyphagous Shot Hole Borer, PSHB) are invasive beetles in North America that vector plant diseases named Laurel wilt and Fusarium dieback, respectively. Both infections are caused by the beetles' fungal symbionts called *Raffaelea lauricola* and *Fusarium euwallaceae*, among others, which block the fluxes of water and nutrients in the plant vascular tissues and cause the death of trees in few weeks. Among the hosts of these pathogenic fungi are avocado, mango, guava and other 200 plant species distributed in more than 50 botanical families. The recent detection of a closely related *Euwallacea* species, the Kuroshio-SHB in Tijuana, Mexico, represents an urgent need to control the spread of the diseases transmitted by these beetle species, which are now considered a national phytosanitary problem. CONACyT (National Council of Science and Technology) through an Institutional Fund for Regional Development for Scientific, Technological and Innovation (FORDECyT) therefore funded to an unprecedented multidisciplinary and interinstitutional research program which main objective is the holistic search of new strategies for the integrated management of these pests (integrating all the agents of the two pathosystems and their interconnections: host, phytopathogen and insect vector) generating cutting-edge actions through different scientific areas such as microbiology, biogeography, chemical ecology, organic chemistry, entomology, biotechnology, plant pathology and omics sciences, among others.

The proposal also includes actors from the productive sector represented by the Producers Association and Avocado Exporters Packers of Mexico (APEAM) and governmental instances such as the Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA) which through the National Service of Health, Safety and AgroFood Quality (SENASICA) and the National Phytosanitary Reference Center (CNRF) will also participate in this project as consultors and facilitators of specific phytosanitary infrastructure. We will present a general perspective of the different strategies that are currently being implemented in this research proposal. Funded by CONACyT-FORDECyT grant (#292399).

Factors that regulate *SPATULA* (*SPT*) expression in *Arabidopsis thaliana*

Angela Guadalupe Juárez-Corona, Víctor Manuel Zúñiga-Mayo and Stefan de Folter*
Unidad de Genómica Avanzada (LANGEBIO), CINVESTAV-IPN
Km. 9.6 Libramiento Norte Carr. Irapuato-León 36824 Irapuato Gto. México Tel: +52 (462) 1663000
*stefan.defolter@cinvestav.mx

The flowers of Arabidopsis develop at the apex of the stem from the inflorescence meristem and each is formed by different floral organs. The gynoecium is the female reproductive organ, which develops from the fusion of two carpels and when fully developed, self-fertilization occurs to obtain the fruit. For the proper development, correct coordination of genetic and hormonal factors is essential. The transcription factor SPATULA (SPT) and the phytohormones auxin and cytokinin have been shown to have an important role in the development of the gynoecium. In *spt* mutants, alterations have been reported in the development of various tissues, resulting in obtaining smaller fruits and reduced seed production. Recent studies have indicated that auxin and cytokinin have a synergistic relationship during gynoecium development, and that SPT is important for this interaction. Until now there is poor information about the transcriptional regulation of *SPT*, therefore, it is important to know the genetic factors and hormones that could be acting in its regulation in tissues of interest such as the gynoecium. In this work, bioinformatic and molecular studies were carried out to broaden the panorama regarding *SPT* regulation. The results obtained indicate that transcription factors belonging to different families are capable of binding to the *SPT* promoter region, suggesting that the transcriptional regulation of this gene could occur through the combinatorial action of different transcription factors. Furthermore, exogenous application of cytokinin and auxin induce the expression of *SPT* in the gynoecium. Both hormones could be acting synergistically in the transcriptional regulation of *SPT*. These results lay the foundation for a detailed analysis about the transcriptional regulation of *SPT*.

Cracking the mysteries of zygotic embryogenesis in avocado by systems biology

Janet Juarez-Escobar[1], Olinda Velázquez López[1], Monica Ramírez Vázquez[1], Lorena López-Sánchez[1], Jose M. Elizalde-Contreras[1], Juan L. Monribot-Villanueva[1], José A. Guerrero-Analco, Víctor M. Loyola-Vargas[2], Martín Mata-Rosas[3], Eliel Ruiz-May[1]
[1]Red de Estudios Moleculares Avanzados y [3]Red de Manejo Biotecnológico de Recursos, Clúster Científico y Tecnológico BioMimic®, Instituto de Ecología A.C. (INECOL), Xalapa, Veracruz, México. Tel. 228 8421800 ext. 6406 Email: martin.mata@inecol.mx y Email: eliel.ruiz@inecol.mx
[2]Unidad de Bioquímica y Biología Molecular de Plantas, Centro de Investigación Científica de Yucatán, Mérida, Yucatán, Mexico.

Angiosperm embryogenesis is a process that begins right after single or double fertilization with the formation of a zygote, that after several cellular divisions and differentiation will become a mature embryo. In general, embryogenesis involves the morphogenic transition through the establishment of a basal cell, a suspensor, and the globular, oblong, heart, torpedo and cotyledonary stage. The understanding of the process of embryogenesis at the structural, biochemical and molecular level forms the basis for comparison with other types of embryo formation, such as somatic, pollen, adventitious embryogenesis as well as the parthenogenetic embryo. However, in avocado, little is known about the transition through the canonic stages of embryogenesis process; the knowledge of it would be useful for improving the current protocols used for *in vitro* regeneration via somatic embryogenesis in avocado, given that the low rate of somatic embryos conversion is the current main bottleneck of this process. The primary goal of this study is to integrate structural features of zygotic embryogenesis acquired with advanced microscopy and massive proteomics and metabolomic massive data. As the first stage in this study, we present preliminary information on confocal microscopy and proteomics data during the zygotic embryogenesis of *Persea americana* Mill var. Hass. The results would give us the insights of avocado development stages and the establishment of a pattern for further determination of the way in which nutrients, hormones, metabolic pathways and other *in vitro* culture factors affect this developmental process.

Keywords: avocado, zygotic embryogenesis, advanced microscopy, proteomics, metabolomics

The effect of synthetic microbial rhizosphere community on the growth of pioneer plants, in mine tailings

Arely Lechuga-Jiménez[1,2]; Diana P. Gallego1, Rocío Cruz-Ortega[2] y Luis David Alcaraz[1]
[1] Laboratorio de Genómica Ambiental, Departamento de Biología Celular Facultad de Ciencias, UNAM y [2] Laboratorio de Alelopatía, Departamento de Ecología Funcional, Instituto de Ecología, UNAM, Circuito Exterior S/N Ciudad Universitaria, Ciudad de México, C.P. 04500 Tel. 5622-9032. Correo electrónico. lalcaraz@ciencias.unam.mx

The mining activity has left unconfined residues known as mine tailings that are characterized by high acidity, no vegetation, lack of organic matter and nutrients, and the presence of potentially toxic elements. In, Nacozari de Garcia, in the state of Sonora, Mexico, there is mine wasteland product of the by the Moctezuma Copper Company, but it has been abandoned for the last 50 years. Native plants were able to colonize the mine tailings, and this helps to stop mine tailings pollution effects due to erosion and dispersion of heavy metals into the Nacozari town which sprawl is now build over the wastelands. We are interested in studying the microbiota of role in pioneering plant establishment and plant growth promotion. We are using the native plant *Acacia farnesiana*, a pioneer plant of mine tailings effects when inoculating an artificial microbial community, isolated from rhizospheres within the mine tailings. The growth of *A. farnesiana* and the synthetic microbial community effect on it was evaluated on the in a greenhouse experiment. Treatments consist of the inoculation of this microbial community in 30-days old after germination plants, using sterile or non-sterile substrate and with presence or absence of mine tailing as controls. The standard analysis of plant growth and the concentration of chlorophyll were used to evaluate vegetable responses. The metagenomic DNA from the synthetic bacterial community was extracted for shotgun sequencing. We are presenting data from the classical analysis of plant growth showing that there were no differences in the stem thickness and spines number were statistically different among treatments. Dry plant biomass was different between treatments, as well as the absolute and relative growth rate. We also measured the concentration of chlorophylls, and there are significant differences between treatments. We are currently evaluating the synthetic community metagenome to find out genes responsible for showing the positive effect observed on plant growth, suggesting that the rhizosphere microbiomes have a role in the establishment of plants in mine tailings.

Identification of candidate genes regulated by ATX1 and involved in root development.

Selene Napsucialy Mendivil, Svetlana Shishkova and Joseph G. Dubrovsky.

Departamento de Biología Molecular de Plantas, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Av. Universidad #2001, Col. Chamilpa, Cuernavaca, Morelos, México. C.P. 62250. Tel. 3291625. Email: snapsu@ibt.unam.mx

In plants, most organs, including roots, are formed during postembryonic development. The *ARABIDOPSIS HOMOLOG OF TRITHORAX1* (*ATX1*) is a known regulator of floral development which encodes a histone methyltransferase (H3K4me). ATX1 is directly involved in the H3K4 trimethylation which is associated with maintenance of transcriptionally active chromatin state. To identify candidate genes regulated by ATX1 and involved in root development, we performed transcriptome sequencing (RNA-Seq) of the *atx1-1*^{setm} mutant root tissues. In this mutant, the ATX1 protein maintains its structural integrity but catalytically is inactive. The mRNA was extracted from roots of 14 days seedlings in two independent experiments. The transcriptome profiling of the root in *atx1-1*^{setm} mutant showed that 355 genes were differentially expressed. Of these, 195 genes were downregulated and 160 were upregulated. To examine the molecular pathways affected in the *atx1-1*^{setm} root, we performed a gene ontology analysis of the differentially expressed genes. Among downregulated genes we found significant enrichments of genes in 'plant cell wall organization' and 'trichoblast differentiation' categories. With this analysis various putative targets of ATX1 were identified. Some of them are the plant-specific transcription factors: *TEOSINTE BRANCHED/ CYCLOIDEA/ PROLIFERATING CELL FACTOR2 and 24*, *AGAMOUS LIKE14*, *LATERAL ORGAN BOUNDARIES DOMAIN*29 and *HOMEOBOX PROTEIN21*. Also we found genes specifically expressed in pericycle that changed their expression in the background of the *atx1-1*^{setm}mutant, among them *PEROXIDASE 35-RELATED, NAC DOMAIN CONTAINING PROTEIN 103* and *PROTODERMAL FACTOR1* which were downregulated in *atx1-1*^{setm} mutant. This suggests that these genes might be directly regulated by ATX1. The RNA-seq data were validated by RT-qPCR analysis of transcript abundance of selected genes. Results of characterization of the mutants in candidate genes regulated by ATX1 will be presented. Research was supported by DGAPA, UNAM (IN200818, IN201318) and CONACyT (237430, 240055).

Lead shortening of primary root share mechanism with phosphate starvation and is independent of the STOP1 pathway

Ortiz-Luevano Ricardo1,2, Valdez-Hernández Mario Fernando1, Flores-Castañea Sandra Azucena1, Martínez-Trujillo Miguel2, Sánchez-Calderón Lenin1*.
1. Laboratorio de Genómica Evolutiva, Unidad Académica de Ciencias Biológicas Universidad Autónoma de Zacatecas. 2. Laboratorio de Microbiología y Genética, Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo.
* Campus II, Av. Preparatoria s/n, colonia Agronómica, C.P. 98066.
Tel. 4921564496; xamachana22@gmail.com; leninsanc@uaz.edu.mx

Plants acquire water and nutrients from the environment through the root system, nevertheless, these resources are not always homogeneously distributed in the soil. Plants modify the post-embryonic development program (PEDP) of their root system to explore the soil, obtain resources or avoid adversities. In soil, in addition to water and nutrients, there are toxic elements such as heavy metals (HMs). In response to HMs toxicity plants modify their root system architecture but not all HMs induces same responses. Some such as arsenic (As) or mercury (Hg) inhibit development and growth of the root system while chrome (Cr) or lead (Pb) promotes the growth of the root system at sublethal concentrations. Pb enhances lateral root density and shortened primary root, similar phenotype has been reported in response to phosphate (Pi) starvation. In media, Pb and Pi react to form an insoluble compound suggesting that the effect of Pb on root phenotype is related to Pi starvation stress. To solve this we analyzed physiological and morphological response seedlings of WT *Arabidopsis thaliana* (Col-0), AtPT1::GUS and AtPT2::GUS (Pi starvation response markers) and mutants (*lpi1, lpi3, lpr1;2 and stop1*) insensitive to Pi starvation in medium supplemented with Pb. First, seedlings were grown in MS 0.1X medium supplemented with Pb(NO3)2 as a source of Pb with increasing concentrations from 0 to 1000µM of Pb. Based on our results we selected concentration of 800 µM due to show a phenotype similar to Pi starvation, short primary root, high lateral root density and exhausted meristems. A characteristic of stress due to Pi starvation is the increase of expression of high affinity phosphate transporters, we contrast response of AtPT1::GUS and AtPT2::GUS to Pi starvation (1µM) and Pb toxicity stress (800µM) we found that GUS staining was lower in Pb treatment than Pi starvation and a smaller percentage of exhausted meristems in Pb treatment of AtPT1 (39% ±6.8) and AtPT2 (32.2% ±5.5) against Pi starvation (76.8% ±5.3 and 86.5% ±3.4 respectively), this results suggest that short primary root and increase of lateral root density phenotype in Pb treatment is not totally due to Pi starvation occasioned by interaction between Pb and Pi in culture medium. Then we found that in 800 µM Pb supplemented medium *stop1* mutants show the same phenotype that Col-0 while *lpr1;2, lpi1*, and *lpi3* were resistant to primary growth inhibition due to Pb, we conclude that unlike the response to Pi starvation which generates primary root growth inhibition by *LPR1* and *ALMT1-STOP1* pathways, in Pb treatments morphogenic response is independent of the *ALMT1-STOP1* pathway. Although *lpi1, lpi3, lpr1;2* primary roots were not shortened in Pb treatment, the gravitropic angle was modified between 10 to 30° toward the left unlike col-0 seedlings, this suggests that *lpi1*, *lpi3*, *lpr1;2* genes are involved in shorten primary root due to Pb toxicity and are necessary to maintain correct gravitropic response in Pb supplemented medium. We conclude that the phenotype of Pb toxicity is not totally due to Pi starvation and share some mechanisms in which are involved *lpi1, lpi3, lpr1;2* and is independent of the *ALMT1-STOP1* pathway.

Absence of BYPASS1 signal aborts the arbuscular mycorrhizal invasion in *Phaseolus vulgaris*

Manoj-Kumar Arthikala[1*], Kalpana Nanjareddy[1], Govindappa M[2], Miguel Lara[3]

[1]Ciencias Agrogenómicas, Escuela Nacional de Estudios Superiores, Universidad Nacional Autónoma de México (UNAM), León, C.P. 37684, Guanajuato, Mexico.
[2]Department of Biotechnology, Dayananda Sagar College of Engineering, Shavige Malleshwara Hills, Kumaraswamy Layout, Bengaluru 560 078, Karnataka, India.
[3]Departamento de Biología Molecular de Plantas, Instituto de Biotecnología, Universidad Nacional Autónoma de México (UNAM), Cuernavaca C.P. 62271, Morelos, Mexico. *Corresponding author: manoj@enes.unam.mx; Tel. 01(477) 194 08 00 Ext.43462

ABSTRACT

BYPASS1 (*BPS1*), which is a well-conserved gene in plants, is required for normal root and shoot development. In the absence of *BPS1*gene function, *Arabidopsis* overproduces a mobile signaling compound (the *BPS1* signal) in roots, and this transmissible signal arrests shoot growth and causes abnormal root development. Recently, in legumes we have demonstrated that BPS1 signal is required for inducing cortical cell divisions during root nodule symbiosis in *Phaseolus vulgaris*. In continuation herein, we explored the role of *Phaseolus vulgaris BPS1* during arbuscular mycorrhizal (AM) fungi symbiosis using an RNA-interference (RNAi) silencing approach. Our results show that in *PvBPS1* downregulated transgenic roots, AM fungi failed to enter the cortex of the roots. At the point of contact with the host roots, the hyphal tips bulged and aborted the hyphal tip growth. Compared to control the appressoria density was significantly low on the *PvBPS1* downregulated root surface. Further the quantitative data of radical hyphal density also found altered. The expression analysis of the *NSP1*, *NSP2*, *RAM1* and *RAM2* were found to be affected in transgenic roots. The promoter::GUS analysis also reveals that the *BPS1* expression is associated with the mycorrhiza colonization. Together, our data show that *PvBPS1* plays a crucial role in the establishment of *Phaseolus-mycorrhizal* symbiosis. This work is supported by PAPIIT (DGAPA-UNAM) grant no. IA205117 to MK.A and IN211218 to K.N.

Proteins implicated during the formation and degradation of Lipid droplets in *Ustilago maydis.*

Minerva Georgina Araiza Villanueva [1], Thomas Pohlmann [2], Michael Feldbrügge [2], Juan Pablo Pardo [3], Meritxell Riquelme [4] and Guadalupe Guerra Sánchez [1*].
[1]Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional. Ciudad de México, México 11340.
[2] Institute for Microbiology, Cluster of Excellence on Plant Sciences, Department of Biology, Heinrich-Heine University Düsseldorf, 40204 Düsseldorf, Germany
[3]Departamento de Bioquímica, Facultad de Medicina, UNAM. Ciudad de México, México 04515.
[4]Departmento de Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Ensenada, Baja California, 22860, México
[*]For correspondence: Email: lupegs@hotmail.com

Lipid droplets (LDs) are organelles that serve as a reservoir of neutral lipids and maintains the lipid homeostasis in the cell. LDs are formed by a core of neutral lipids surrounded by a monolayer of phospholipids and proteins such as perilipin and seipin, which are related with the formation and maintenance of LDs. Otherwise it has been reported that cytosolic lipases control the homeostasis between synthesis and degradation of triacylglycerols (TAGs). Under nitrogen limitation, *Ustilago maydis* forms LDs; although the dynamics of this organelles are described, there is no information about the role of perilipin, seipin and cytosolic lipases during the formation and degradation of LDs. To investigate this, we deleted seipin and perilipin in *U. maydis* FB2 by homologous recombination following the Golden Gate method. The wild type (wt) FB2 as well as the mutant strains of perilipin and seipin in FB2 were characterized following their growth in YPD and minimal media without nitrogen. Also, the phenotype of those strains was observed by confocal microscopy, using BODIPY to dye the LDs. For lipase assay, we determinated the lipid droplet index and lipase activity; and traced the cytosolic lipases that are implicated in the mobilization of LDs accumulated under nitrogen starvation, by a zymogram. Preliminary results showed that when wild type cells and mutants were grown in minimal medium with glucose as carbon source, wt cells had a higher growth rate than the mutants. The deletion of perilipin and seipin also affected the morphology and number of LDs in *U.* maydis under nitrogen starvation. The mutant of perilipin formed smaller LDs which did not increase their size, in comparison with the wt LDs. The mutant of seipin had few lipid droplets the first day, but after 48h the cells lost their LDs. Otherwise we demonstrated that accumulated lipids are degradated by lipases and these are used in the growth process. We observed that formation and degradation of LDs are complementary processes. This is the first report about *U. maydis* cytosolic lipases involved in LDs degradation.

Changes of Acrosomal pH During Human Sperm Capacitation

Gabriela Carrasquel Martínez and Alberto Israel Darszon.
Departamento de Genética del Desarrollo y Fisiología Molecular. Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, México 62210.
Phone: +5217773291611. Electronic address: gabycm@ibt.unam.mx

In the female reproductive tract mammalian sperm must undergo a series of biochemical and physiological modifications, collectively called capacitation, before the Acrosome Reaction (AR) occurs. This exocytotic reaction is essential for fertilization. During capacitation, intracellular calcium and pH increases are necessary to promote AR. Our group has shown that membrane permeable weak bases, such as some blockers of CatSper (a sperm-specific, pHi dependent, Ca2+ channel) are able to increase acrosomal pH (pHa) and trigger the AR (Chávez et al., 2018. J Cell Physiol. 233:4735). These findings indicate that pHa regulation may play an important role in the AR. Nevertheless, in human spermatozoa it is unknown what happens to the pHa during capacitation, nor which channels or transporters present in the acrosome membrane are regulated by pHa. In this work

we evaluated pHa changes during capacitation of human sperm. Spermatozoa recovered by swim-up from healthy donors were capacitated for 6 h and their pHa measured at 0, 3 and 6 h of incubation. LysoSensor green was used to determine pHa by single cell epifluorescence microscopy and flow cytometry with image analysis (AMNIS). The results showed that pHa progressively increases during capacitation, the change being significant after 6 h of incubation (p□0.005). As anticipated, inhibition of the acrosomal V-ATPase induced a sustained pHa alkalization during capacitation which reached higher values than those obtained in the absence of the inhibitor. Our results show that during human sperm capacitation pHa is alkalized, contributing to the preparation of the sperm to undergo AR. The cytoplasmic alkalization mediated by V-ATPase play an important role in the regulation of pHa.

Keywords: Acrosome, acrosomal pH, Human Capacitation.

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Actin cytoskeleton dynamics during human sperm capacitation

Valeria Castillo-Viveros[1], Paulina Torres-Rodríguez[1], Alberto Darszon[1], Mariano G. Buffone[2] and Claudia L. Treviño[1]
[1]Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México.
[2]Instituto de Biología y Medicina Experimental (IBYME), Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Buenos Aires, Argentina.

Cuernavaca, Morelos 62210, +52 (777) 329-1611 ctreviño@ibt.unam.mx

Actin is an indispensable component of the cytoskeleton and plays a crucial role in many cell types. The actin cytoskeleton is essential in the generation and maintenance of cell morphology, motility, and exocytosis. It has been proposed that the actin cytoskeleton in mammalian sperm participates in a maturation process known as capacitation that takes place during sperm transit in the female tract. Sperm capacitation is a requisite to acquire the ability to fertilize the egg. Nevertheless, the actin dynamic changes that occur during capacitation have not been studied in live sperm. The main reason is the lack of membrane permeable probes and the difficulty of using genetically encoded actin reporters in sperm cells.

Recently, a silicon-rhodamine dye (SiR-Actin) derived from the toxin Jasplakinolide has been introduced. SiR-Actin is a membrane-permeable fluorescent probe that binds actin *in vivo*. In an effort to understand the function of the actin cytoskeleton in human sperm during capacitation, we used SiR-Actin to analyze actin dynamics in non-capacitated and capacitated sperm using image-based flow cytometry. This novel technique permits the acquisition of fluorescence images from thousands of cells allowing a reliable quantitative analysis even in different sperm regions.

Sir-Actin had not been used in human sperm before, therefore we first established the experimental conditions to use this probe. *In vitro* capacitation mimics the female tract environment to promote capacitation incubating sperm with media supplemented with BSA and HCO3- for a period of time. We followed actin dynamics in sperm incubated in different capacitation conditions (complete capacitation medium, or medium lacking either BSA or HCO3-). Regardless of the media condition, the incubated sperm showed a time dependent increase in actin polymerization. Consistently, when capacitated sperm cells were incubated with Latruncilin A, an inhibitor of the actin polymerization, we did not observe the increment in actin polymerization. Analysis of sperm subcellular regions indicates that during capacitation the middle and the principal pieces, as well as the head, had an increment in actin polymerization, the latter being the largest.

These results support the notion that actin polymerization takes place during sperm capacitation. However, further studies are needed to clarify the role of this process in sperm maturation and its relation to the ability to fertilize the oocyte.

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Image-based flow cytometry as a tool to study rheotaxis in human spermatozoa

Mariana Beatriz Olivares Urbano[1, 2], Arturo Matamoros Volante[1] , Paulina Torres Rodriguez[1], Alberto Darszon[1] and Claudia L. Treviño Santa Cruz[1‡]
[1]Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, 62250, Cuernavaca, Morelos,
[2]Licenciatura en Ciencias,Centro de Investigación en Dinámica Celular, Universidad Autónoma del Estado de Morelos, 62250,Cuernavaca, Morelos, México. [‡]ctrevino@ibt.unam.mx

Sexual intercourse in mammals induces the secretion and flow of fluid along the female genital tract. It has been proposed that sperm can be directed and guided by this flow because their ability to swim against it (process known as rheotaxis). Some reports indicate that mammalian spermatozoa rheotaxis depends of on the capacitation state of the cells, a post-ejaculation maturation process that takes place during transit in the female reproductive tract. On the other hand, some authors suggest that rheotaxis is a passive process which depends only on sperm morphology.

In an effort to better understand sperm rheotaxis, in this work, we have explored the use of image-based flow cytometry to analyze this behavior in human sperm. This technique allows multi-parametric analysis of large cell populations like in conventional flow cytometry, with the advantage of an optical system that records an image of every cell being analyzed. As the equipment generates a fluid flow in order to align the cells towards the interrogation point, we wondered if spermatozoa would orient themselves against the flow generated by equipment, and we could study rheotaxis and determine if the orientation depends on the sperm capacitation state.

To analyze the data, we developed a novel strategy to localize the position of the sperm head in the image and automatically determine if the cell is orientated with or against the flow.

We incubated human spermatozoa in non-capacitating and capacitating conditions for 1, 2 and 4 hours. As a negative control, samples from each incubation time were killed by a heat-shock before acquisition. Preliminary results show that cells alive display a preferential orientation against the flow which increases with capacitation time. In contrast, dead cells display a random orientation behavior with roughly 50% in each direction.

Further research and controls are required to establish that image-based flow cytometry is a reliable technic to study sperm rheotaxis and examine if this process is capacitation dependent.

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Expression of Septins 1 and 2 during the Oogenesis of *Aedes aegypti*

José Ángel Rubio Miranda, Uriel Enrique Aquino Ruiz, Antonio Celestino Montes, Leticia Cortés Martínez, Febe Elena Cázares Raga, Fidel de la Cruz Hernandez-Hernandez. Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional. Av. Instituto Politécnico Nacional No. 2508. Col. San Pedro Zacatenco C.P. 07360 México, ciudad de México.
cruzcruz@cinvestav.mx

Introduction: *Aedes aegypti* mosquitoes are vectors of arbovirus causing major health problems in Mexico: Dengue, Zika and Chikungunya and the study of its biology is important because this knowledge can be used for the design of novel vector control strategies, without insecticides, that could be efficient and environment friendly. Reproduction and sex determination are two main research fields for this aim. Oogenesis is the process in which germline stem cells become eggs. In *Drosophila melanogaster*, as the best dipteran model, it has been described that oogenesis begins with the formation of a cyst of 16 cells from the germ line, which are interconnected and one of them is the oocyte, which will mature and become an egg. Septins are proteins that participate in diverse cellular processes, such as cytokinesis, vesicular traffic, chromosomal segregation, cellular polarity, apoptosis and their participation in the oogenesis and the transformation of the syncytial to cellular blastoderm of *D. melanogaster* has also been observed. Mutations in different septin can affect the maturation of the oocyte, causes abnormal ovarioles with deformed follicular cells surrounding the oocyte, or can stop the embryo development.
Objective: In the present work the expression and cellular localization of Septins 1 and 2 during the process of oogenesis in *Aedes aegypti* was analyzed.
Material and Methods: Eggs of *Aedes aegypti*, Rockefeller strain, were hatched in water, larvae stages were harvested for the experiments or cultured until adult stage. For the analysis of oogenesis, ovaries were dissected from bloodfed female mosquitoes, at 12, 24, 36, 48 and 60 hours after feeding. With the purpose of analyzing expression and celular localization of Septins 1 and 2 during oogenesis of *A. aegypti* the dissected ovaries were subjected to RNA extraction, cDNA generation, PCR and immunofluorescence assays, using heterologous commercial antibodies directed against mammalian septins which sequences were highly homologous to putative septin sequences recovered from *A. aegypti* genome databases using BLAST search.
Results: During the process of oogenesis in *A. aegypti*, septins 1 and 2 increased their expression, at 12, 24 and 36 hours of development and decreasing between 48 to 60 hours. Regarding cellular localization, at 12, 24 and 36 hours, septins are located in the cytoplasm of the egg chamber and after 48 and 60 hours, these are found only in the periphery of the follicular cells surrounding the egg chamber.
Discussion: Expression of septins 1 and 2 was demonstrated experimentally in *A. aegypti* tissues, these proteins change their expression during egg maturation in *A. aegypti*, similarly to *D. melanogaster*, suggesting they are of important for the correct development of the egg, and that the interference of its expression could be a strategy to decrease the reproduction of this mosquito.

A predicted cargo adaptor, CNI, plays an important role in traffic of specific essential material for the growth of *Neurospora crassa*

Luis Enrique Sastré-Velásquez* and Meritxell Riquelme
Departamento de Microbiología.
Centro de Investigación Científica y de Educación Superior de Ensenada, CICESE
Ctra. Ensenada-Tijuana N.3918. Ensenada, B.C. México. 22860.
+52 646 107 78 72 *
lsastre@cicese.edu.mx *

Hyphae of filamentous fungi extend by apical polarized growth. This phenomenon is supported by the transport of secretory vesicles directed to the apex, where the accumulation of macro and microvesicles gives rise to the Spitzenkörper (SPK). These vesicles provide the plasma membrane and the cell wall with all the molecular components necessary to expand. The secretory pathways ensure the traffic of molecules to different cellular destinations. The coat protein complex II (COPII) is involved in the formation of COPII secretory vesicles from the endoplasmic reticulum (ER). Some of the proteins involved in COPII vesicles formation include activators of this complex, the inner and outer vesicle coat proteins and different cargo adaptors, such as the transmembrane proteins belonging to the Cornichon/Erv14 family. *D. melanogaster* Cni is required for the efficient export of the growth factor TGF Gurken (Grk), involved in oogenesis. *S. cerevisiae* Erv14 is the specific cargo adaptor for some proteins, such as Axl2, required to establish polarized growth and axial budding.
In this work, we identified a putative orthologue of Cornichon/Erv14 in *Neurospora crassa* (CNI; NCU06922) and analyzed its localization in growing hyphae and relative to the main organelles previously characterized in this fungus. CNI tagged with GFP was found at very dynamic puncta along the hyphae and close to the tip, moving primarily in an anterograde manner. Co-expression of CNI-GFP and CSE-7-mChFP, previously identified as a putative cargo adaptor for CHS-4, revealed a partial co-localization of both proteins in some distinct regions of the NEC (Network of Elongated Cisternae). CSE-7 was found all along the NEC, whereas CNI was delimited to punctate zones of the NEC, suggesting that CNI localized at the ER exit sites (ERES) of the NEC. CNI-GFP and mChFP-YPT-1, a Rab GTPase previously identified at early and late Golgi cisternae and at the core of the SPK, co-localized partially at some of the Golgi cisternae. This localization suggests that CNI buds off from the ERES of the NEC embedded in vesicles, in their way to release specific cargoes after fusion with early Golgi cisternae. Co-expression of the COPII putative component SEC-24 labeled with mChFP and CNI-GFP, showed partial co-localization of both proteins, corroborating the participation of CNI in COPII vesicles formation. This study identifies CNI as a putative cargo adaptor at ERES and early Golgi cisternae. In the long term we aim to provide some light onto the importance of cargo-adaptors in the development of filamentous fungi.

Aplicaciones de microfluidica para el análisis de células individuales.

La célula se considera la unidad fundamental de la función biológica. Durante mucho tiempo se consideró que existe una cierta homogeneidad en poblaciones celulares pequeñas y se han llevado a cabo estudios de muestras celulares basados en esta hipótesis. Esto ha dado lugar a que los resultados obtenidos presenten promedios de las características analizadas sin tomar en cuenta que cada célula es única y que sus propiedades pueden cambiar de manera diferente a los estímulos a los que está sujeta.

Por lo anterior en años recientes ha habido un amplio desarrollo de técnicas para llevar a cabo estudios con un nivel de resolución de células individuales. Este enfoque permite detectar respuestas y características celulares que no son detectables cuando se analizan poblaciones celulares, por ejemplo, detección de células raras como las células cancerosas circulantes o células residuales asociadas a enfermedades.

Entre las técnicas que se han aplicado al aislamiento de células para permitir análisis con resolución de célula individual esta la microfluidica, técnica que ha mostrado contar con algunas características importantes en el estudio de células individuales, tales como preservar la integridad bilógica, permitir cuantificación o aislar una gran cantidad de células en poco tiempo con gran eficiencia.

En esta presentación llevaremos a cabo una introducción a la técnica y ejemplos de aplicación en transcriptómica e inmunología entre otras, así como las opciones de equipos que Dolomite Bio ofrece para el encapsulamiento de células y haremos una breve mención de otras posibles aplicaciones de los sistemas de microfluidos en el desarrollo de formas farmacéuticas para la liberación controlada de fármacos.

AUREUS SOLUCIONES INTEGRALES PARA LABORATORIO S.A.DE C.V.
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Cell Mutiparameter Analysis using Fluorescence Microscopy
High-content analysis – Automated imaging and analysis of cellular assays

Sandra Rosa da Silva, Msc
Product Group Manager - Latin America
GE Healthcare Life Sciences

What's the future of your cellular research?

Cellular research traditionally involves analysis techniques such as biochemical assays, microscopy, Western blotting, and flow cytometry. While these techniques are highly valuable to the researcher they can have low throughput, involve labor-intensive processes, and destroy the cells being analyzed. This puts constraints on the number and complexity of assays that can be handled and limits the number of questions that can be asked in the research, particularly where a high number of assays are required, for example in RNAi studies.
Recent advances in high-throughput automated microscopy, now commonly referred to as high-content analysis (HCA), mean researchers are using these traditional techniques in combination with newer, more informative cellular assays. The automation and throughput provided by HCA allows you to ask questions that you would not get the opportunity to ask with other techniques, and to answer them more quickly.

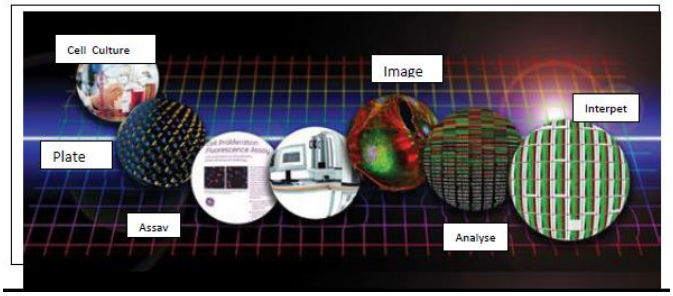
Why use HCA?
The benefits of HCA apply to a wide variety of cellular assays including cell signaling, toxicology, RNAi knockdown, cell differentiation and morphology, cell cycle, neurology, protein trafficking, and receptor activation. However, with the increased use of HCA in mainstream cellular research, the possibilities of the technology are constantly expanding. Whatever your cellular research involves, HCA can offer that extra dimension:

Increase throughput and productivity:
Assays typically take much less time than traditional techniques such as Western blotting and light microscopy enabling you to generate more data for publication.

Improve data quality:
Single cell analysis in multiple wells enables you to easily quantitate results, run multiple controls for increased data confidence, and produce statistically relevant data in a fraction of the time typically taken manually.

Investigate in breadth:
Test hypotheses rigorously by correlating results from other techniques with effects observed and measured directly in the cell.

Investigate in depth:
Automated imaging of non-destructive multiplexed live cell assays allows you to follow complex signaling events as they happen and also measure proteins, DNA, RNA, and cell morphology in a single assay.



Evolucionando el Western blot a través de la multidetección fluorescente

Carlos Bravo

Life Science Group, Bio-Rad México. carlos_bravoalberto@bio-rad.com

Western blot es una importante técnica para detección de proteínas, muy utilizada por biólogos celulares y moleculares en todo el mundo. Ha permanecido vigente por casi 40 años sin sufrir cambios importantes en su metodología. Con el desarrollo de nuevas tecnologías, Bio-Rad ha revolucionado y mejorado una técnica aumentando su robustez y confiabilidad. Haciendo uso de diversas técnicas fluorescentes y la normalización por proteína total, el llamado “V3 Western Workflow” se introdujo como una herramienta valiosa para el análisis de expresión proteica. Un flujo de trabajo enfocado en disminuir significativamente el tiempo del proceso, incrementar la reproducibilidad e introducir la validación de resultados cuantitativos. En la actualidad, esta tecnología en conjunto con el uso de la detección de fluorescencia multiplex a través de anticuerpos “StarBright” han permitido la identificación de hasta 3 distintas proteínas simultáneamente. Conoce más sobre las ventajas y beneficios de la evolución en el Western Blot.

Technologies and information were generated by Life Science Group, Bio-Rad Laboratories, Inc.

Edición de Genomas
M. en C. Carlos Humberto Martínez Paniagua,
Gerente del departamento de soporte científico de UNIPARTS

A pesar de existir metodologías disponibles para llevar a cabo edición de genomas, como los *zinc fingers* y TALENS, estas cuentan con una capacidad limitada para llevar a cabo de forma precisa y rápida las ediciones de genoma. La tecnología CRISPR/Cas9 representa un gran avance sobre otras metodologías, teniendo de esta manera una metodología que mejoran la eficiencia y la especificidad de ensayos de edición de genomas, además de ser una técnica relativamente sencilla de realizar. El sistema CRISPR/Cas9 aumenta la especificidad de edición en el blanco deseado en prácticamente todos los organismos y tipos celulares.

En esta platica se hace una descripción de la metodología CRISPR/Cas9, componentes, consideraciones de diseño, así como un flujo de trabajo experimental típico de este tipo de ensayos.

“Nuevas herramientas para el estudio del epigenoma en humano y ratón por secuenciación de siguiente generación”

Dr. Alejandro Xchel Rivera González.

Científico de Aplicaciones, Agilent Technologies México.

Resumen: La metilación del ADN es probablemente uno de los fenómenos epigenéticos mejor caracterizados y hoy en día está claramente establecido que en asociación con otros factores juega un papel clave en la regulación de la expresión génica. Con la llegada de las tecnologías de secuenciación masiva del ADN se tiene la oportunidad de estudiar este fenómeno epigenético con un nivel de detalle sin precedentes. Sin embargo, a pesar de que estas nuevas tecnologías permiten la secuenciación completa del metiloma, la complejidad del análisis y la infraestructura necesaria para realizar este tipo de experimentos suele ser una limitante en muchos laboratorios. Por esta razón, las metodologías de enriquecimiento de blancos se ha vuelto una alternativa popular para el estudio exclusivo de una porción definida del metiloma. Teniendo esto en cuenta, Agilent Technologies ha desarrollado “Methyl-seq”, una herramienta que permite la captura y secuenciación de regiones específicas del metiloma de interés clínico-biológico. Experimentos llevados a cabo en nuestra plataforma con tejidos de humano y ratón muestran que, Methyl-seq permite la eficiente identificación de regiones diferencialmente metiladas usando solamente 1 µg de de ADN como muestra inicial y 10 Gb (80 millones de lecturas) de secuenciación por muestra. Los análisis de las métricas de secuenciación demuestran que Methyl-seq alcanza una cobertura del 90 % de las bases blanco con una profundidad de al menos 10X y una uniformidad del 91 %. Finalmente, para ilustrar la utilidad de esta herramienta, llevamos a cabo un pequeño experimento en ratones en donde se les administró una dosis de 100 µg/mL de Corticosterona por cuatro semanas. Al término del tratamiento el grupo tratado y su respectivo control fueron sacrificados y muestras de sangre y tejido cerebral fueron obtenidas para extracción de ADN. Las muestras fueron procesadas usando la herramienta Methyl-seq y finalmente encontramos aproximadamente 5,000 regiones diferencialmente metiladas entre el grupo tratado contra el grupo control. Por último, validamos la metilación diferencial de tres de estas regiones usando piro-secuenciación y encontramos una concordancia del 100 % con respecto a lo observado en la secuenciación. En conclusión, el contenido de la herramienta Methy-seq se enfoca a regiones con relevancia clínico-biológica, permitiendo la identificación de regiones diferencialmente metiladas con una resolución de un solo nucleótido. Además, al enfocarse en regiones específicas del metiloma permite incrementar fácilmente el número de muestras sin sacrificar desempeño y calidad de los datos.