SMB XXXI

# CONGRESO NACIONAL DE BIOQUÍMICA

## 06 - 11 de Noviembre 2016

## Aguascalientes

Fecha límite de inscripciones y envío de resúmenes:

**30 de junio**

**Comité Organizador**

Miguel Lara Flores

Irene Castaño Navarro

Guadalupe Espín

Jorge Luis Folch

**Sede:** Hotel Marriot

**Informes:**
nacional@smb.org.mx

## www.smb.org.mx

SMB · Facultad de Química, Universidad Nacional Autónoma de México · Academia Mexicana de Ciencias · Instituto de Fisiología Celular · CCG Centro de Ciencias Genómicas · Coordinación de la Investigación Científica · Instituto de Biotecnología, Universidad Nacional Autónoma de México · CONACYT · Cinvestav · RELAB Red de Ciencias Biológicas Latinoamericana · Secretaría de Turismo · OCV Aguascalientes

**XXXI Congreso Nacional de Bioquímica**

**6-11 Noviembre, 2016**

**Aguascalientes, Ags.**

# *Programa*

**Comité Organizador y**
**Mesa Directiva 2015 - 2017**

**Dr. Miguel Lara Flores**
Presidente

**Dra. Irene Beatriz Castaño Navarro**
Vicepresidente

**Dra. Guadalupe Espín Ocampo**
Secretaria Tesorera

**Dr. Jorge Luis Folch Mallol**
Subsecretario

**Edición Técnica:** María Teresa Castillo
**Diseño Portadas:** Servicios y Formas Gráficas
**Soporte Técnico:** Andrea Ortiz, Diana Cordero, Omar Chávez

**XXXI Congreso Nacional de Bioquímica**

**6 al 11 de noviembre, 2016 Aguascalientes, Ags**

*Mensaje de la Presidente*

Estimados Colegas:

A todos los participantes en el XXXI Congreso Nacional de la Sociedad Mexicana de Bioquímica, les damos las gracias y la más cordial bienvenida. Este es un evento que representa la continuidad de esfuerzos, sueños e ilusiones de estudiantes, profesores, investigadores, jóvenes y adultos. Todos en comunión, por un mayor y mejor desarrollo de las ciencias bioquímicas en México.

Es para la mesa directiva y para mí como presidente de la Sociedad Mexicana de Bioquímica, una gran distinción y un honor el participar y dar continuidad a esta tan generosa labor que inició hace más de 60 años, de impulsar y estimular a la comunidad científica en las áreas de la bioquímica.

Si revisamos el perfil de los participantes en este congreso, podemos constatar que participan 44 Instituciones de educación superior, 12 centros de investigación y 13 institutos de salud pública del país. Además contamos con la participación de 14 instituciones extranjeras. Es sin lugar a duda, un evento con una alta representatividad de la comunidad científica-académica de México. Con la distinguida presencia de colegas lideres académicos, de otros países, el Congreso nos da la opción de establecer colaboraciones internacionales y así contar con un mayor potencial científico.

Quiero señalar muy enfáticamente, que del total de los participantes, el 72% son estudiantes tanto de posgrado como de licenciatura y 28% investigadores. En gran medida, esto representa la razón de ser de estas reuniones. Veamos con entusiasmo este congreso, en donde los jóvenes que inician hoy sus presentaciones, serán en el futuro cercano, los académicos consagrados, de igual forma que muchas generaciones lo han hecho en todos estos años de existencia de la Sociedad Mexicana de Bioquímica.

Que este texto además de ser un mensaje de bienvenida, sea una invitación a hacer de este Congreso el foro por excelencia, en el que los jóvenes puedan comunicar sus resultados y enriquecerse con las observaciones y comentarios de los investigadores experimentados, que sea también la plataforma de donde despeguen las colaboraciones futuras que cohesionen y enriquezcan a esta comunidad.

*Miguel Lara Flores*
Presidente SMB 2015-2017

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

*Al Instituto de Fisiología Celular, UNAM*
Por todas las facilidades prestadas a la Sociedad Mexicana de Bioquímica

*A la Oficina de Convenciones y Visitantes de Aguascalientes*
Por su apoyo prestado para la logística del congreso

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

*Al Equipo de Trabajo:*
*Biól. Andrea Ortiz Arcos, Lic. Diana Verónica Cordero Tavares, Ricardo Chávez Castillo, Omar Chávez Castillo y María Teresa Castillo*
Por la gran labor que han realizado. Por haber puesto todo su esfuerzo, dedicación y entusiasmo en cada una de las etapas del congreso. Por su compromiso incondicional para con la Sociedad Mexicana de Bioquímica, a todos ellos agradecemos el logro alcanzado.

**Table of Contents**



## Instructivo de navegación

**XXXI CONGRESO NACIONAL DE BIOQUÍMICA** **Aguascalientes, Ags. November 6-11, 2016**

| SUNDAY 6 | | MONDAY 7 | TUESDAY 8 | WEDNESDAY 9 | THURSDAY 10 | FRIDAY 11 |
|---|---|---|---|---|---|---|
| REGISTRATION<br>11:00 – 18:00<br><br>DURING ALL<br>THE MEETING | 8:30 – 10:30 | Plenary Lecture<br>**EVOLUTIONARY IMPLICATIONS OF HYBRIDIZATION IN FUNGI**<br>Toni Gabaldón<br><br>Plenary Lecture<br>**THE PHYSIOLOGY AND HABITAT OF LUCA: THE LAST UNIVERSAL COMMON ANCESTOR**<br>William F Martin | Plenary Lecture<br>**DNA TOPOLOGY AND THE REGULATION OF TRANSCRIPTION**<br>David Levens<br><br>Plenary Lecture<br>**NEURAL CODING OF SUBJECTIVE SENSORY EXPERIENCE AND UNCERTAINTY OF PERCEPTUAL DECISIONS**<br>Ranulfo Romo | Plenary Lecture<br>**INFLUENZA VIRUS-HOST INTERACTIONS**<br>Adolfo Garcia Sastre<br><br>Plenary Lecture<br>**MTOR AND REGULATION OF CELL GROWTH**<br>John Blenis | Plenary Lecture<br>**THE CONTROL OF EUKARYOTIC PROTEIN SECRETION BY A NOVEL PATHWAY REGULATING GLUTATHIONE TRAFFIC IN AND OUT OF THE ENDOPLASMIC RETICULUM**<br>Michel Toledano<br><br>Plenary Lecture<br>**MITOCHONDRIAL BIOENERGETICS AND OXPHOS SUPERCOMPLEXES**<br>Oscar Flores | PLENARY SESSIONS<br>-EPIGENETICS<br>-COMPUTATIONAL BIOLOGY |
| | 10:30 - 11:00 | | COFFEE BREAK | | | |
| | 11:00 – 13:30 | CONCURRENT SESSIONS<br>-BIOTECHNOLOGY<br>-BASIC BIOCHEMISTRY<br>-GENETIC, EPIGENETIC & GENETIC REGULATION | CONCURRENT SESSIONS<br>-MICROBIOLOGY & VIRUSES<br>-BASIC BIOCHEMISTRY<br>- GENETIC, GENETIC REGULATION & EPIGENETIC | CONCURRENT SESSIONS<br>- IMMUNOLOGY & PARASITOLOGY<br>- MICROBIOLOGY & VIRUSES<br>- SIGNAL TRANSDUCTION & CELL DIFFERENTIATION<br>-Technical Conferences | CONCURRENT SESSIONS<br>- SYSTEMS BIOLOGY & BIOINFORMATIC<br>- BIOTECHNOLOGY<br>- GENETIC, GENETIC REGULATION & EPIGENETIC | CONCURRENT SESSIONS<br>-SYSTEMS BIOLOGY & BIOINFORMATIC<br>-BASIC BIOCHEMISTRY<br>- IMMUNOLOGY & PARASITOLOGY |
| | 13:30 – 15:30 | LUNCH | LUNCH | LUNCH | LUNCH | LUNCH |
| | 15:30 – 18:00 | PLENARY SESSIONS<br>-PROTEIN, STRUCTURE & FUNCTION<br>-NEURAL PLASTICITY: FROM MOLECULES TO CIRCUITS<br>-FUNGAL MOLECULAR BIOLOGY | PLENARY SESSIONS<br>- NEW APPROACHES ON THE STUDY OF BACTERIA<br>- BIOCHEMISTRY AND PLANT MOLECULAR BIOLOGY<br>- SIGNAL TRANSDUCTION | FREE TIME | PLENARY SESSIONS<br>-OXIDATIVE STRESS IN LIFE<br>-BIOENERGETIC<br>-EMERGING VIRUSES | POSTER SESSION<br>-IMMUNOLOGY AND PARASITOLOGY<br>-NEUROSCIENCES AND NEUROLOGY<br>-TOXICOLOGY AND PHARMACOLOGY<br>-SIGNAL TRANSDUCTION<br>**15:30 – 17:30** |
| OPENING CEREMONY<br>17:00<br><br>Opening Lecture<br>**SCIENCE ACCORDING TO FEDERICO SANCHEZ: ABOUT STAYING YOUNG AS A SCIENTIST**<br>Herman Spaink<br>17:30 – 18:30<br><br>Plenary Lecture<br>**STRUCTURAL BIOLOGY OF TGF-BETA FAMILY SIGNALING PROTEINS NEW INSIGHTS INTO MECHANISM TO NOVEL THERAPIES FOR CANCER AND FIBROSIS**<br>Andrew P Hinck<br>Protein Design<br>18:30 – 19:30 | 18:00 – 20:00 | POSTER SESSION<br>-SYSTEMS BIOLOGY & BIOINFORMATICS<br>-BASIC BIOCHEMISTRY<br>-OXYGEN REACTIVE SPECIES | POSTER SESSION<br>-BIOTECHNOLOGY<br>-MEDICINE, HEALTH AND NUTRITION | | POSTER SESSION<br>-GENETIC, EPIGENETIC AND GENETIC REGULATION<br>-MICROBIOLOGY AND VIRUSES | Plenary Lecture<br>**EPIGENETICS AND ONCO-METABOLITES**<br>Jasper Rine<br>**17:30 – 18:30** |
| WELCOME COCKTAIL<br>19:30 | | | BUSINESS SESSIONS- SMB<br>20:00 | | | CLOSING DINNER<br>21:00 |

# *Program of Events*

Sunday November 6, 2016

11:00 – 18:00 Registration
Hotel Marriott

**Opening Ceremony, Plenary Lectures**

San Marcos Room

17:00 – 17:30 Opening Ceremony

17:30 – 18:30 **Opening Lecture**
Science according to Federico Sanchez: about staying young as a scientist
**Herman Spaink**
Leiden University
"*In Memoriam* Dr. Federico Sánchez Rodríguez"

Chair: *Miguel Lara Flores*
Instituto de Biología, UNAM

18:30 – 19:30 **Plenary Lecture**
Structural biology of TGF-beta family signaling proteins – new insights into mechanism to novel therapies for cancer and fibrosis
**Andrew P. Hinck**
University of Pittsburgh School of Medicine

Chair: *Luis Brieba de Castro*
LANGEBIO CINVESTAV Unidad Irapuato

19:30 – 21:30 Welcome Cocktail
Garden Hotel Marriott

Oral presentations will be held in the San Marcos Room, Hotel Marriott

Poster presentations will be held in the Garden, Hotel Marriott

Monday November 7, 2016

**8:30 – 10:30 Plenary Lectures**

8:30 – 9:30 Evolutionary implications of hybridization in fungi
**Toni Gabaldón**
ICREA Research Professor, Comparative Genomics Group, Centre for Genomic Regulation (CRG), Barcelona, Spain

Chair: *Irene Castaño*
IPICYT

9:30 – 10:30 The physiology and habitat of LUCA: the last universal common ancestor
**William F. Martin**
Heinrich HeineUniversität Düsseldorf, Germany

Chair: *Guadalupe Espín*
Instituto de Biotecnología, UNAM

10:30 – 11:00 Coffee break
Foyer San Marcos Room

**Concurrent Sessions**

Monday November 7, 2016

| 11:00 – 13:30 | **1. Biotechnology**<br><br>**San Marcos One Room** | **2. Basic Biochemistry**<br><br>**San Marcos Two Room** | **3.Genetic, Epigenetic & Genetic Regulation**<br>**San Marcos Three Room** |
|---|---|---|---|
| | Chair: *Ana Paulina Barba*<br>IPICyT | Chair: *Gloria Yepiz*<br>CIAD Hermosillo | Chair: *Norma Silvia Sánchez*<br>IFC UNAM |
| 11:00-11:20 | **Effect of PH on *E. Coli* producer of a phospholipase A2 from *Micrurus laticollaris* snake venom.** *Carlos Calcines Cruz*, Mauricio A. Trujillo Roldán, Alejandro Olvera, Alejandro Alagón Norma A. Valdez Cruz. Instituto de Investigaciones Biomédicas, UNAM | **Change in protein-ligand specificity through statistical coupling analysis.** *Jesús A. Banda Vázquez*,Sooruban Shanmugaratnam, Rogelio Rodríguez Sotres, Alfredo Torres Larios, Birte Höcker, Alejandro Sosa Peinado. Facultad de Medicina**,** UNAM | **Glucose regulates lifespan through a network of stress-responsive transcription factors in *Caenorhabditis elegans.*** *Jonathan Alcántar Fernández*, Rosa Estela Navarro González, Ana María Salazar Martínez, Martha Elva Pérez Andrade, Juan Miranda Ríos. Instituto de Investigaciones Biomédicas, UNAM |
| 11:20-11:40 | **Biodegradation of Diclofenac by the native fungi *Pleurotus djamor* isolated from Chiapas** *Rosbi Cruz Ornelas*, Lorena Amaya Delgado, Griselda Karina Guillén Navarro, José Ernesto Sánchez Vázquez, María de los Ángeles Calixto Romo. Colegio de la Frontera Sur | **Comparative study of transport and assimilation of xylose in conventional and non-conventional yeasts** *Alejandra Karina Estrada Ávila***,** Antonio Peña Díaz, Juan Carlos González Hernández, Alicia González Manjarrez. Instituto de Fisiología Celular,UNAM | **Microarray Analysis of differentially expressed genes in trophoblast with iodine deficiency.** *Arroyo Helguera Omar Elind*, Xochihua Rosas Irene, Jiménez González Azalia, and Garduño Gabriel. INSP, Universidad Veracruzana |
| 11:40-12:00 | **The Defensin from Avocado (*Persea americana var. Drymifolia*) padef Induces Apoptosis in the Leukemia Cell Line K-562.** *Luis José Flores Álvarez*, Rodolfo López Gómez, Alejandra Ochoa Zarzosa, Joel Edmundo López Meza. Universidad Michoacana de San Nicolás de Hidalgo | **Analysis of the Redox Components of Bacillus subtilis cytochrome $b_6c$ Complex. The Activity of a Menaquinol: cytochrome *c* Reductase.** *Ana Paula García García*, Emma Berta Gutiérrez, Cirlos Madrid. FES Iztacala, UNAM | **Co-regulation of CSD1 and ADH1 mRNAS by miR398 and miR2119 in response to stress in *Phaseolus vulgaris.*** *Carlos De la Rosa*, Alejandra A. Covarrubias, José Luis Reyes. Instituto de Biotecnología, UNAM |
| 12:00-12:20 | **Soil and rhyzospheric bacteria with inhibition of root rot causing pathogens in chili pepper, with differential SAR related gene induction in plant: nine partially sequenced genomes.** *Saúl Fraire Velázquez*, Martínez Raudales Inés, De La Cruz Rodríguez Yumiko, Alvarado Gutiérrez Alejandro, Vega Arreguín Julio. Universidad Autónoma de Zacatecas | **Biological Activity or extracts *Tournefortia spp*. Cell Line MCF-7 breast cancer and HeLa of cervical cancer.** *Laura Pérez Campos*, Zoila Mora Guzmán, Luis Ángel Laguna Barrios, Ruth Martínez Cruz, Rebeca López Marure, Eduardo Pérez Campos, Ma. Del Socorro Pina Canseco. Instituto Tecnológico de Oaxaca | **Novel mechanisms of lifespan extension revealed by genome-wide screening of dietary-restriction factors in yeast.** *Sergio Esteban Campos Rodríguez***,** Erika Garay, Alejandro Juárez Reyes, Alexander de Luna. LANGEBIO. CINVESTAV. Irapuato |

| | 1. Biotechnology<br><br>San Marcos One Room | 2. Basic Biochemistry<br><br>San Marcos Two Room | 3.Genetic, Epigenetic & Genetic Regulation<br>San Marcos Three Room |
|---|---|---|---|
| | Chair: *Ana Paulina Barba.*<br>IPICyT | Chair: *Gloria Yepiz*<br>CIAD. Hermosillo | Chair: *Norma Silvia Sánchez*<br>IFC UNAM |
| 12:20-12:40 | **Oxygen transfer rate affects undecylprodigiosin synthesis in *Streptomyces lividans*: relation with recombinant glycoprotein production.** *Ramsés A. Gamboa Suasnavart*, Luz D. Marín Palacio, Norma A. Valdez Cruz, Wolf Klöckner, Jochenbüchs and Mauricio A. Trujillo Roldán. Instituto de Investigaciones Biomédicas, UNAM | **17-DMAG disturb the subcelular and extracellular localization between Hsp90α and Hsp90β and determines the cell migration capacity in cervical cancer cells.** *Morales Guadarrama Silvia Gabriela*, José de la Luz Díaz Chávez, Alejandro López Saavedra, Luis Alonso Herrera Montalvo, Carlo César Cortés González. Investigación Biomédica en Cáncer. IIB. UNAM | **Genetic and genomic analysis of zygotic genome activation in *Arabidopsis.*** *Gerardo Del Toro de León***,** Daoquan Xian, Cei Abreu Goodger, Raju Datla, Stewart Gillmor. LANGEBIO. CINVESTAV. Irapuato |
| 12:40-13:00 | **Engineered Cytochrome P450 BM-3 reductase domain stabilized by consensus mutagenesis.** *Valeria Guzmán Luna*, Gloria Saab Rincón, Hanan Alwaseem, Rudi Fasan, Frances H. Arnold Instituto de Biotecnología, UNAM | **Semi-automatized analysis using image-based flow cytometry to study capacitation-associated increase in tyrosine phosphorylation in human sperm.** *Arturo Matamoros Volante*, Ayelen Moreno Laura Giojalas, Claudia L.Treviño. Instituto de Biotecnología, UNAM | **Local DNA topology is a selection factor that determines the association of DNA to the nuclear matrix.** *David García Vilchis***,** Armando Aranda Anzaldo. Facultad de Medicina, Universidad Autónoma del Estado de México |
| 13:00-13:20 | **Some like it hot: Biomolecular analytics using MicroScale Thermophoresis**. *Dinorah Leyva*. NanoTemper Technologies do Brasil ltd | ***Moringa oleifera* leaf extract preserves mitochondrial respiratory activity in HepG2 cells exposed to hyperglycemia.** *Jorge Alejandro Sosa Gutiérrez*, Mónica Valdez Solana, Maurizzio Battino, Alfredo Téllez Valencia, Claudia Domínguez Avitia, Gonzalo García Vargas, Oscar Flores Herrera, José Salas Pacheco, Erick Sierra Campos. Universidad Juárez del Estado de Durango | **Npa3/ScGpn1 carboxy-terminal domain is dispensable for cell viability and RNA polymerase II nuclear targeting but critical for microtubule stability and function** *Gehenna Lobo Guerrero Serrano*, Leonardo Castañedo, Gema R. Cristóbal Mondragón, Javier Montalvo Arredondo, Lina Riego Ruiz, Alexander De Luna, Alejandro De Las Peñas, Irene Castaño, Mónica R. Calera, Roberto Sánchez Olea. Instituto de Física, Universidad Autónoma de San Luis Potosí |

13:30 – 15:30 Lunch

**Plenary Session 1**
Monday November 7, 2016
15:30 – 18:00 San Marcos Room I

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**Protein Structure and Function**

Chair: *Luis Brieba de Castro*
LANGEBIO CINVESTAV Unidad Irapuato

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15:30 – 16:00 Structural biology of organellar replisomes
Noe Baruch, Alma Fuentes, Antolin Peralta, Annia Rodriguez, Carlos Trasviña, María E Sánchez, Corina Díaz Quezada, Alfredo Torres Larios and **Luis G. Brieba**
LANGEBIO CINVESTAV Unidad Irapuato

16:00 – 16:30 Roles of Non-Catalytic Residues in the Activity and Regulation of Plant ALDH10 Enzymes
**Rosario Muñoz Clares**
Departamento de Bioquímica, Facultad de Química. UNAM

16:30 – 17:00 tRNA loading by divergent pathways
Marco Igor Valencia Sánchez, Annia Rodríguez Hernández, Rubén Ferreira, Hugo Aníbal Santamaría Suárez, Marcelino Arciniega, Anne Catherine Dock Bregeon, Dino Moras, Brice Beinsteiner, Haydyn Mertens, Dmitri Svergun, Luis G. Brieba, Morten Grøtli, and **Alfredo Torres Larios**
Departamento de Bioquímica y Biología Estructural, Instituto de Fisiología Celular, UNAM

17:00 – 17:30 Protein characterization and biological model development from mass spectrometry data
**Robert Winkler**
CINVESTAV Unidad Irapuato

17:30 – 18:00 Biophysical approaches to studying the regulation of gene expression
**Kristina Marie Herbert**
Departamento de Microbiología. Centro de Investigación Científica y de Educación Superior de Ensenada

**Plenary Session 2**
Monday November 7, 2016
15:30 – 18:00 San Marcos Room II

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**Neural plasticity: from molecules to circuits**

Chair: *Angélica Zepeda Rivera*
Instituto de Investigaciones Biomédicas, UNAM

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15:30 – 16:00 Cellular plasticity during neuronal differentiation and in the adult brain.
**Luis Fernando Covarrubias Robles**, Dulce María Arzate Vázquez, Gilda Guerrero Flores, Ana Karen Mojica Ávila, Gladys Mondragón Figueroa, Magdalena Guerra Crespo, Omar Collazo Navarrete
Instituto de Biotecnología, UNAM

16:00 – 16:30 Synaptic and homeostatic plasticity orchestrating the persistence of memory
**Martha L. Escobar Rodríguez**
División de Investigación y Estudios de Posgrado, Facultad de Psicología. UNAM

16:30 – 17:00 Neurogenesis and sexual behavior
**Raúl G. Paredes** and Wendy Portillo
Instituto de Neurobiología UNAM

17:00 – 17:30 Run for your neurons! Physical exercise and adult neurogenesis
**Carmen Vivar**
Laboratory of Neurogenesis and Neuroplasticity. Department of Physiology, Biophysics and Neuroscience, CINVESTAV

17:30 – 18:00 Neurogenesis as a potential mechanism of repair after brain damage
**Angélica Zepeda Rivera**
Instituto de Investigaciones Biomédicas, UNAM

**Plenary Session 3**
Monday November 7, 2016
15:30 – 18:00 San Marcos Room III

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**Fungal Molecular Biology**

Chair: *Alfredo Herrera Estrella*
LANGEBIO CINVESTAV Irapuato

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15:30 – 16:00 Suppression of the extracellular ATP (eATP) triggered host defense response by the root endophytic fungus *Piriformospora indica*
Shadab Nizam and **Alga Zuccaro**
Max Planck Institute for Terrestrial Microbiology &University of Cologne, Germany

16:00 – 16:30 MAP kinase dynamics during cell-cell communication and fusion in *Neurospora crassa*
**André Fleißner**
Institut für Genetik, Technische Universität Braunschweig, Germany

16:30 – 17:00 The MAPK signaling pathways: new roles in fungal physiology
**Edgardo Ulises Esquivel Naranjo** and Alfredo Herrera Estrella
Facultad de Ciencias Naturales. Universidad Autónoma de Querétaro

17:00 – 17:30 Phospholipid flippases and vesicle traffic during polarized growth
**Rosa R. Mouriño Pérez**, Ivan Murillo Corona, Zachary Schultzhaus and Brian D. Shaw
Department of Microbiology. Centro de Investigación Científica y Educación Superior de Ensenada

18:00 – 20:00 Poster Session 1

SB SYSTEMS BIOLOGY & BIOINFORMATICS
B BASIC BIOCHEMISTRY
OR OXYGEN REACTIVE SPECIES

## SYSTEMS BIOLOGY & BIOINFORMATICS

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|---|---|
| **SB-1** | **Annotation, phylogeny and quantitative RNA sequencing of the bean AUTOPHAGY family genes in mycorrhizal symbiosis.** *Jessica Monserrat Aguilar*, Kalpana Nanjareddy, Miguel Lara and Manoj Kumar Arthikala. Escuela Nacional de Estudios Superiores – León. UNAM |
| **SB-2** | **Global expression profiling reveals genetic networks associated with growth and development in TOR down regulated *Phaseolus vulgaris* roots**. *Alma Leticia Aguirre*, Kalpana Nanjareddy, Miguel Lara and Manoj KumarArthikala. Escuela Nacional de Estudios Superiores – León. UNAM |
| **SB-3** | **Evolutionary dynamics of microRNA regulatory networks**. *Gilberto Alejandro Álvarez* Canales**,** Luis Delaye, Cei Abreu Goodger. LANGEBIO, CINVESTAV Irapuato |
| **SB-4** | **Genomic analysis of bacterial DNA methylation and repair systems**. *Dagoberto Armenta Medina*, Carmen Gómez Eichelmann, Cei Abreu Goodger. Departamento de Biología. CINVESTAV Irapuato. |
| **SB-5** | **Revealing metagenomic microbial diversity using reference pan-genomes**. *Hugo Rafael Barajas de la Torre*, Luis David Alcaráz. Instituto de Ecología, UNAM |
| **SB-6** | **Testing the tomato root's microbiome assembly rules.** *Hugo Rafael Barajas de la Torre*, Luis David Alcaráz. Instituto de Ecología, UNAM |
| **SB-7** | **Species interactions mediated by small RNAs**. *José Roberto Bermúdez Barrientos*, Cei Abreu Goodger. LANGEBIO CINVESTAV Irapuato |
| **SB-8** | ***De novo* transcriptome sequencing by RNA-seq of an Asteraceae species to identify genes involved in the biosynthesis of secondary metabolites**. *Génesis Vidal Buitimea Cantúa*, Enrique Ramírez Chávez, Juan Vázquez Martínez, Jorge Molina Torres. CINVESTAV IPN Irapuato |
| **SB-9** | **Cell death pathways *in silico* model based on biochemical tuple spaces for self-organizing coordination**. *Lilia Karina Cabrera Cosme*, Maura Cárdenas García, Pedro Pablo González Pérez. Autonomous University of Puebla, Cell Physiology Laboratory, School of Medicine |
| **SB-10** | **Identification of Transcription Factor Binding Sites AraC/XylS-Type in *Escherichia coli* K-12 using a new Phylogenetic Footprinting pipeline**. *Gieraldin Campos Lozada*, Patricia MR Oliver Ocaño, María L Tabche Barrera, Teresa Romero Cortes, Víctor H Pérez España, Enrique Merino Pérez and Peralta Gil Martin. Instituto Tecnológico Superior del Oriente del Estado de Hidalgo |
| **SB-11** | **Systematic analysis of lifespan-epistasis effects in the budding yeast *Saccharomyces cerevisiae***. *Erika Viridiana Cruz Bonilla*, Alexander de Luna Fors. CINVESTAV Irapuato |
| **SB-12** | **Structural analysis of MrpA subunit from Na+/H+ antiporter complex of the archaeon *Methanosarcina acetivorans***. *César Díaz Pérez*, Alma Laura Díaz Pérez, Rafael Alejandro Veloz García, María Isabel García Vieyra, Patricia Castro Moreno, José Salud Rodríguez Zavala, Ricardo Jasso Chávez. Departamento de Ingeniería Agroindustrial. Campus Celaya. Universidad de Guanajuato |
| **SB-13** | **Deciphering potential determinants for exosomal miRNAs package**. *Ricardo de Jesús Ehecatl Gómez Reyes*, Kristina Marie Herbert. CICESE |
| **SB-14** | **Arbuscular mycorrhiza symbiosis induces a major transcriptional reprogramming of the common bean nutrient transporter families**. *Brenda Mariana Gómez*, Kalpana Nanjareddy, Miguel Lara and Manoj Kumar Arthikala. Escuela Nacional de Estudios Superiores, Unidad León. UNAM |
| **SB-15** | **Designing of a bioinformatic system for the 16S ribosomal sequences massive analysis**. *Everardo Gutiérrez Millán*, Gabriel Guillén Solís, Rosaura Aparicio Fabre, Claudia Díaz Camino. UPEMOR |
| **SB-16** | **Effect of stimulation with interleukin-10 on peripheral blood mononuclear cells: a bioinformatic analysis**. *Juan Manuel Guzmán Flores,* Edgar Iván López Pulido, Saúl Ramírez De los Santos. Centro Universitario de los Altos, Universidad de Guadalajara |
| **SB-17** | **Environmental gene-content homoplasy and evolution of *Bacillus***. *Ismael L Hernandez Gonzalez***,** Gabriel Moreno Hagelsieb, Gabriela Olmedo Alvarez. Departamento de Ingeniería Genética. CINVESTAV Irapuato |
| **SB-18** | ***De Novo* transcriptome assembly of telomerase-negative strains of *Ustilago Maydis***. *José Juan Jacinto Vázquez***,** Candelario Vázquez Cruz, Estela Anastacio Marcelino, Guillermo Manuel Horta Valerdi, Ma. Patricia Sánchez Alonso. Instituto de Ciencias. BUAP |

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| **SB-19** | **Phylogenetic analysis of the chromate ion transporter (CHR) superfamily revisited**. *Adriana Julián Sánchez***,** José Manuel Contreras Sánchez, Rosa I. Reyes Gallegos, Carlos Cervantes, Héctor Riveros Rosas. Departamento Bioquímica. Facultad de Medicina, UNAM |
| **SB-20** | **Comparative analysis of the protease sequence of *Totoaba macdonaldi***. *Ana Paola López Reyes Guerrero***,** Manuel Ignacio Carretas Valdez, Aldo Alejandro Arvizu Flores. Departamento de Investigación y Posgrado en Alimentos, Universidad de Sonora |
| **SB-21** | **Genome assembly and comparative genomics analysis of Ca. *Mycoplasma haemobos* strain INIFAP01, the first hemotrophic mycoplasma identified in Mexico**. *Fernando Martínez Ocampo***,** Rosa Estela Quiroz Castañeda, Luis Fernando Lozano Aguirre Beltrán, Sergio Dario Rodríguez Camarillo, Itzel Amaro Estrada. CENID Parasitología Veterinaria. INIFAP |
| **SB-22** | **V2 promoter variant in silico analysis of human gene *ST3Gal4***. *Monterrosas Santamaría José Ricardo***,** Vallejo Ruíz Verónica, Reyes Leyva Julio, Milflores Flores Lorena. Escuela de Biología. BUAP |
| **SB-23** | **Constraint-based modeling in cervical cancer, a multiomics approach**. *Felipe Muñoz Gonzalez***,** Osbaldo Resendis Antonio. Facultad de Medicina. Instituto Nacional de Medicina Genómica |
| **SB-24** | **Extracellular small RNAs during parasite-host communication**. *Cesaré Ovando Vazquez***,** Franklin Chow, Georgios Koutsovoulos, Tuhin Maity, Mark Blaxter, Julie Claycomb, Amy Buck, Cei Abreu Goodger. LANGEBIO CINVESTAV Irapuato |
| **SB-25** | **Molecular dynamics studies of CssIV scorpion β-toxin binding to the voltage gated Nav1.2**. *Ana Estela Pérez Mejía***,** José Luis Velasco Bolom, Ramón Garduño Juárez. Instituto de Ciencias Físicas, UNAM |
| **SB-26** | **Analysis of the dynamic of deletions in mitochondrial DNA from human somatic cells**. *Bertha Guadalupe Rueda Zarazúa***,** Alfredo Varela Echavarría, Jorge Tonatiuh Ayala Sumuano. Instituto de Neurobiologia. UNAM |
| **SB-27** | **Transcriptomic analysis of the halophile adaptation responses of *Aspergillus caesiellus* in a lignocellulosic solid-state fermentation**. *María del Rayo Sánchez Carbente***,** Yordanis Pérez Llano, Jorge Luis Folch Mallol, Ramón Batista García. Centro de Investigacion en Biotecnologia, Universidad Autónoma del Estado de Morelos |
| **SB-28** | **Functional conservation of neuronal microRNAs**. *Gabriela Santos Rodríguez***,** Cei Abreu Goodger. LANGEBIO. CINVESTAV Irapuato |
| **SB-29** | **Transcriptional modification of Fma1, Fma2 y Pca1 genes in the model of chronological aging of *Schizosaccharomyces pombe*.** *Patricia Leany Segundo Ibáñez***,** Olaf Rosas Galicia, Lourdes Millán Pérez Peña, Irma Herrera Camacho, Nora Hilda Rosas Murrieta. Instituto de Ciencias. BUAP |
| **SB-30** | **Genomic sequencing of *Herbaspirillum* sp. strain TQ07 and characterization of the metabolic pathway involved in chloranilic acid degradation**. *Luis Gerardo Treviño Quintanilla,* Itzel López Mendoza, Juan José Colín Salinas. Universidad Politécnica del Estado de Morelos |
| **SB-31** | **Pore-forming mechanism of the antimicrobial peptide Pandinin-2**. *José Luis Velasco Bolom*, Ramón Garduño Juárez. Instituto de Ciencias Físicas, UNAM |
| **SB-32** | **BIK Interacts with coding and non-coding regions of the Breast Cancer MDA-MB-231 cells**. *Miguel Ángel Velázquez Flores***,** Karen Contreras Ayala, Juan Manuel Rodríguez Corona, Javier Torres López, Ruth Ruiz Esparza Garrido. Centro Médico Nacional Siglo XXI. IMSS |

## BASIC BIOCHEMISTRY

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| **B-1** | **Towards an understanding of the mechanism of a microtubule-length regulator: the kinesin-8 Kip3**. *Hugo Arellano Santoyo*. Dana Farber Cancer Insitute/Harvard Medical School. Howard Hughes Medical Institute |
| **B-2** | **Crystallization of copper chaperon LVATX from *Litopenaeus vannamei* (Penaeidae) in binding to different metals**. *Arisbeth Guadalupe Almeida Juárez***,** Enrique Rudiño Piñera. Instituto de Biotecnología, UNAM |

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| **B-3** | **Possible existence of microsomes in which takes place the degradation of polycyclic aromatic hydrocarbons**. *Jazmin Areli Álvarez Copado***,** Roberto Zazueta Sandoval. Universidad de Guanajuato |
| **B-4** | **Effect of the pH on the capacity of iron-binding of chaperonin GroEL from *Helicobacter pylori*.** *Carlos Alberto Alvarez Librado***,** Alma Lidia Olivares Cervantes, Marco Antonio González López, Octavio Daniel Reyes Hernández, Norma Velázquez Guadarrama, Elisa Irene Azuara Liceaga, José de Jesús Olivares Trejo. Posgrado de Ciencias Genómicas. Universidad Autónoma de la Ciudad de México |
| **B-5** | **Design of aptamers for the identification of human pathogen bacteria**. *Carlos Alberto Alvarez Librado***,** Dan Israel Zavala Vargas, María Elizabeth Álvarez Sánchez and José de Jesús Olivares Trejo. Posgrado de Ciencias Genómicas. Universidad Autónoma de la Ciudad de México |
| **B-6** | **Identifying structural determinants of tranglycosidation function in the alpha-amylase enzyme family through residue contact analysis**. *Rodrigo Arreola Barroso***,** David Y. Salgado Rinquey, Gloria Saab Rincón. Instituto de Biotecnología, UNAM |
| **B-7** | **Following the initial contacts of a mitochondrial protein: the NAC complex and the outer membrane proteins Sam37, Om14 and Tom70**. *María Clara Avendaño Monsalve,* Ponce Rojas J. Carlos, Torres Quiroz Francisco, Jaimes Miranda Fabiola, Funes Soledad. Departamento de Genética Molecular, Instituto de Fisiología Celular, UNAM |
| **B-8** | **Study of the effect of cytokinin-auxin crosstalk in somatic embryogenesis of *Coffea canephora***. *Johny Avilez Montalvo*, José Cetz Chel, Geovany Nic Can, Ariana Pérez Hernández, Ángela Kú Gónzalez, Rosa Galaz Ávaloz, Víctor M. Loyola Vargas. Centro de Investigación Científica de Yucatán AC |
| **B-9** | **Biochemical characterization *in vitro* of two novel organelar DNA polymerases of plants (POPs) from *Arabidopsis thaliana***. *Noé Baruch Torres,* Corina E Díaz Quezada, Luis G Brieba. LANGEBIO, CINVESTAV |
| **B-10** | **Involvement of the Bcp1 protein in maturation of the pre-ribosomal 60S subunit, in the ribosomal biogenesis pathway in the yeast *Saccharomyces cerevisiae***. *Arnulfo Bautista Santos,* Nuria Victoria Sánchez Puig. Instituto de Química, UNAM |
| **B-11** | **HSL esterases in fungi? Bioinformatic and biochemical characterization**. *Ramón Alberto Batista García*, María del Rayo Sánchez Carbente, Ayixón Sánchez Reyes, Angela Escudero Garcia, Catalina Morales Herrera, Laura Inés Cuervo Soto, Leidys French Pacheco, Arline Fernández Silva, Carlos Amero, Edmundo Castillo and Jorge Luis Folch Mallol. UAEM |
| **B-12** | **Comparative proteomics of amaranth species: Divergence in seed storage proteins and starch synthesis enzymes.** *Esaú Bojórquez Velázquez***,** Alberto Barrera Pacheco, Eduardo Espitia Rangel, Alfredo Herrera Estrella, Ana Paulina Barba de la Rosa. IPICyT |
| **B-13** | **Biophysical properties of a new allelic variant of triosephosphate isomerase found in human prostate cancer cell line**. *Peniel Bustamante Villalobos,* Valeria Guzmán Luna, Leticia Olvera Rodríguez, Gloria Saab Rincón. Univ. Juárez del Edo de Durango. Instituto de Biotecnología, UNAM |
| **B-14** | **The Formation of the *Supercomplex* $b_6c$:$caa_3$ when cytochrome $c_{550}$ is Overexpressed in *Bacillus subtilis*.** *Tecilli Cabellos Avelar,* Emma Berta Gutiérrez Cirlos Madrid. Biomedical Unit Laboratory, FES Iztacala. UNAM |
| **B-15** | **Effects of cationic molecules on *Candida albicans*.** *Martha Calahorra Fuertes***,** Norma Silvia Sánchez, and Antonio Peña. Instituto de Fisiología Celular, UNAM |
| **B-16** | **Effect of the flavonoid Morin on the cell cycle in an *in vitro* model of pharynx cancer.** *Mariana Camacho Santana***,** Gloria Gutiérrez Venegas. Facultad de Odontología, UNAM |
| **B-17** | **Diversification of the kinetic properties of yeast NADP-glutamate-dehydrogenase isozymes proceeds independently of their evolutionary origin and contributes to the acquisition of fermentative or respiratory lifestyles.** *José Carlos Campero Basaldúa,* Héctor Quezada, Lina Riego Ruiz, Dariel Márquez, Erendira Rojas, James González and Alicia González. Instituto de Fisiología Celular, UNAM |
| **B-18** | **CD43 promotes tumor cells survival, contributing to tumor growth.** *Alicia Cañas Linares***,** Flores Alcantar Ángel, Vega Mendoza Daniela, RosensteinYvonne. Instituto de Biotecnología, UNAM |
| **B-19** | **Trypsin III Mutant A233N of Monterey sardine (*sagax caerulea*): biochemical characterization.** *Manuel Ignacio Carretas Valdez***,** Sergio Casas Flores, Francisco Javier Cinco Moroyoqui, Marina Josafat Ezquerra Brauer, Enrique Márquez Ríos, Rogerio Rafael Sotelo Mundo, Aldo Alejandro Arvizu Flores. Universidad de Sonora |

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| **B-20** | **Over-expression, Purification and Crystallization of Novel Homologues of Adenine Glycosylase MutY with [4Fe-4S] Cluster Dispensability.** *Atzimba Y. Castro Lara,* Carlos H. Trasviña Arenas, Corina Díaz Quezada and Luis Brieba de Castro. LANGEBIO, CINVESTAV Irapuato |
| **B-21** | **Characterization of two mutants in the elongation factor like GTPase (Efl1) and its possible implication in the Shwachman Diamond Syndrome**. *Montserrat Chacón Flores***,** Arnulfo Bautista Santos, Jesús Pérez Juárez, Nuria Sánchez Puig. Instituto de Química, UNAM |
| **B-22** | **Profile genetic evaluation of Th1/Th2/Th17 cytokines in left ventricle of rat during physiological cardiac hypertrophy**. *José Francisco Chipres Montaño***,** Jesús A Rosas Rodríguez, Edgar F Morán Palacio, José F Muñoz Valle, José Gpe. Soñanez Organis. Universidad de Sonora |
| **B-23** | **Structural study of the extended spectumβ-lactamase TLA-1 by X-ray crystallography.** *Víctor H. Cifuentes Castro***,** Jesús Silva Sánchez, Enrique Rudiño Piñera. Instituto de Biotecnología, UNAM |
| **B-24** | **The enzymes of the arginine metabolism and its activity in red blood cells.** *Martha Lucinda Contreras Zentella***,** Pablo Rangel Silva, Rolando E. Hernández Muñoz. Instituto de Fisiología Celular, UNAM |
| **B-25** | ***Ols F*expression in *Burkholderia andropogonis* increase environmental stress tolerance**. *Luz América Córdoba Castro***,** Christian Sohlenkamp. Centro de Ciencias Genómicas, UNAM |
| **B-26** | **Silencing of the HIF-1α affect the glucose and lactate concentrations, and G6PDH activity in shrimp infected with the WSSV**. *Paola María Covarrubias Coronado***,** Leticia A. Encinas Osuna, José Arquimides Godoy Lugo, Jesús A. Rosas Rodríguez, Luis A. Gámez Alejo, Silvia Gómez Jiménez, José Gpe. Soñanez Organis. Ciencias Químico Biológicas y Agropecuarias, Universidad de Sonora |
| **B-27** | **Energetics basis of allosteric inhibition of ABL tyrosine kinase involved in chronic myeloid leukaemia**. *Roberto Cruz Castañeda***,** Mario Trejo, Enrique García Hernández, Axel Luviano. Instituto de Química, UNAM |
| **B-28** | **Study of the physical interaction between the essential GTPases Gpn1 and Gpn3**. *Gema Rosa Cristóbal Mondragón*, Víctor De la Rosa Jiménez, Ernesto Ladrón de Guevara, Gisela E Rangel, Yescas León D Islas Súarez, Roberto Sánchez Olea, Mónica R Calera Medina. Instituto de Física. Universidad Autónoma de San Luis Potosí |
| **B-29** | **Determination of Kinetic Parameters of Trypsin I from Pyloric Caeca of Monterey Sardine (*Sardinops sagax caerulea*) Using Isothermal Titration Calorimetry.** Idania Quintero, Enrique Velázquez, Javier Castillo, Rocío Sugich, *Dalia Cruz*. Universidad de Sonora |
| **B-30** | **Evaluating an assessment of biochemistry and molecular biology**. *Héctor Javier Delgadillo Gutiérrez***,** Yolanda Saldaña Balmori. Departamento de Sistemas Biológicos. Universidad Autónoma Metropolitana |
| **B-31** | **Induction, purification and biochemical characterization of a pectinase from *Ophiostoma piceae***. *Blanca Azucena Delgado Baeza***,** Julio César Villagómez Castro. Departamento de Biología. Universidad de Guanajuato |
| **B-32** | **Protocol of expression and purification of recombinant mitocondrial DNA Polymerase from *Saccharomyces cerevisiae***. *Corina E. Díaz Quezada***,** Eugenia Sánchez Sandoval, Annia Rodríguez Hernández, Luis G. Brieba. LANGEBIO. CINVESTAV. Irapuato |
| **B-33** | ***Yersinia* pseudotuberculosis OppA protein chaperone activity on the α-glucosidase enzyme**. *Escobar Garduño Elena***,** Soto Urzúa Lucía, Scior Thomas, Baca Beatriz Eugenia, Martínez Morales Luis Javier. Facultad de Ciencias Químicas. Universidad Autónoma de Puebla |
| **B-34** | **Differential protein expression in gastric premalignant lesions and gastric cancer by iTRAQ labeling-based proteomics approach.** *Diana Lashidua Fernández Coto***,** Jeovanis Gil Valdés, Ivone Castro Romero, Eva Hernández Márquez, Sergio Encarnación Guevara, Ana Silvia Arenas Linares, A. Hernández, Roberto Herrera Goepfert, Valia Calderon Sosa, Guadalupe Ayala. Instituto Nacional de Salud Pública |
| **B-35** | **Spectroscopic characterization of metal binding to AtLEA4-5 peptides.** *Leidys French Pacheco***,** Alejandra Covarrubias Robles, César Cuevas Velázquez, Carlos Amero Lina Rivillas Acevedo. Centro de Investigaciones Químicas. Universidad Autónoma del Estado de Morelos |
| **B-36** | **Enzymatic synthesis, structural and functional characterization of Primase-polimerasa (PRIM-POL) of *Arabidopsis thaliana.*** *Alma Yazmín Fuentes Pascacio***,** Corina E. Díaz Quezada, Luis G. Brieba. Unidad de Genómica Avanzada. CINVESTAV. Irapuato |

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| **B-37** | ***In vitro* evaluation of elongases (ELOVL 1-7) expression on insulin sensible and resistant adipocytes.** *Esmeralda Cruz Mecate*, Teresa García Gasca, Anaguiven Avalos Soriano, Víctor Javier Sánchez Arévalo Lobo, Ulisses Moreno Celis. Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro |
| **B-38** | **Expression and purification the plant-specific ssDNA binding proteins: mtSSBs, OSBs and Why, from *Arabidopsis thaliana*.** *Paola Libertad García Medel*, Noé Baruch Torres, Corina Díaz Quezada, Luis Gabriel Brieba de Castro. LANGEBIO CINVESTAV. Irapuato |
| **B-39** | **Functional complementation of the ribosomal EFL1 GTPase domains**. *Daniel Said García Montalvo*, Alfonso Méndez Godoy and Nuria Sánchez Puig. Instituto de Química, UNAM |
| **B-40** | **Expression of recombinant Pearlin of *Pinctada fucata* in *Escherichia coli.*** *Lizbeth García Villegas*, Rayana Ruiz Arellano, Enrique Rudiño Piñera. Universidad Autónoma del Estado de Morelos. IBT-UNAM |
| **B-41** | **Biochemical characterization of DARPins with a single binding module.** Mercedes Cervantes López, Diego Nájera Benavides, Daniela Rodríguez, Sergio Enríquez Flores, Ignacio De la Mora De la Mora, Cynthia Fernández Lainez, Gabriel López Velázquez, Gloria Hernández Alcántara, *Itzhel García Torres*. Instituto Nacional de Pediatría |
| **B-42** | **NMR dynamic basis of the EphA-SAM domain complexes**. *Paloma Gil Rodriguez,* Soon Jueng Kim and Matthias Buck. Department of Physiology and Biophysics, Case Western Reserve University |
| **B-43** | **Functional and Biochemical Characterization of Three Recombinant Human Glucose-6-Phosphate Dehydrogenase Mutants: Zacatecas, Vanua-Lava and Viangchan.** *Saúl Gómez Manzo*, Abigail González Valdez, Víctor Martínez Rosas, Beatriz Hernández Ochoa, Erick Alcaraz Carmona, Yadira Yazmín Cortés Morales, Estefania Millán Ramírez de Arellano, Laura Eloisa Morales Luna, Karina Pérez Nuñez, Edson Jiovany Ramírez Nava, María Fernanda Trejo Martínez, Jaime Marcial Quino. Instituto Nacional de Pediatría |
| **B-44** | **The mitochondrion of sea urchin sperm contains the soluble adenylyl cyclase.** *Juan Pablo González Mora*, Alberto Darszon, Ma. del Carmen Beltrán Núñez. Departamento de Bioquímica, UNAM |
| **B-45** | **Phospholipases A2 isolated from centipede venoms**. *Lidia González Morales*, Lorena Sánchez Espinos, Leo Giovani Lezama Urrutia. Universidad Autónoma del Estado de Morelos |
| **B-46** | **Apoptotic effects of Luteolin, Naringin and Quercetin in Head and Neck Squamous Cell Carcinoma cells.** *Ricardo González Salguero*, Gloria Gutiérrez Venegas. Facultad de Odontología, UNAM |
| **B-47** | **Differences on the conformational substates visited by native and mutants versions of the LAO-BP obtained by Accelerated Molecular Dynamics.** *Diego Sebastián Granados Villanueva*, Jesús Banda Vázquez, Alejandro Sosa Peinado. Departamento de Bioquímica, Facultad de Medicina. UNAM |
| **B-48** | **Effect of constant IGF-I administration on insulin and its modulation by biotin.** *Laura Patricia Guerrero Carrillo*, Ana L Vázquez Jiménez, Cecilia Palmas Bustamante, Georgina Díaz Herrera, Jorge Omar García Rebollar, Nancy Y Mora Pérez, Margarita Gómez Chavarín, Armida Báez Saldaña. Institute de Investigaciones Biomédicas, UNAM |
| **B-49** | **Thioredoxin-glutathione reductase (TGR) is the main disulfide reductase expressed in the free-living platyhelminth *Dugesia dorotocephala***. *Alberto Guevara Flores*, José de Jesús Martínez González, I. Patricia del Arenal Mena, Juan Luis Rendón Gómez. Facultad de Medicina. UNAM |
| **B-50** | **Phosphorylation Sites on the Free Fatty Acids Receptor 1, FFA1 (GPR40).** Alejandro Guzmán Silva, María Teresa Romero Ávila, Jesús Adolfo García Sáinz. Instituto de FisiologíaCelular, UNAM |
| **B-51** | **The role of three-pyruvate kinases present in *Vibirio cholerae***. *Gloria Hernández Alcántara*, Carlos Guerrero Mendiola, José J. García Trejo and Leticia Ramírez Silva. Facultad de Medicina, UNAM |
| **B-52** | **Study of the mechanisms underlying enzyme release from hepatic and lung tissues to the extracellular milieu in rats subjected to 70% partial hepatectomy.** *Lorena Carmina Hernández Espinosa*, Lourdes Sánchez Sevilla, Martha Lucinda Contreras Zentella, and Rolando Hernández Muñoz. Instituto de Fisiología Celular, UNAM |
| **B-53** | **Recovery of abundant chondrocytes after differentiation of sheep bone marrow CD90+-mesenchymal stem cells mobilized to peripheral blood and expanded in culture medium with defined fetal bovine serum.** *Cecilia Hernández Flores*, René Valdés Mijares, Carlos Landa Solís, Carmina Ortega Sánchez, J Clemente Ibarra Ponce de León, Rebecca E Franco Bourland. Instituto Nacional de Rehabilitación |

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| **B-54** | **Studies of Molecular Docking of Nicardipine and Nitrendipine on Aldose Reductase.** *Zurisaddai Hernández Gallegos***,** Luis Enrique Montes Vega, Mauro Manuel Martínez Pacheco, Asdrubal Aguilera Méndez, Daniel Godínez Hernández. Universidad Michoacana de San Nicolás de Hidalgo |
| **B-55** | **Dehydrin profile comparation in three maize landraces under drought stress.** *Alejandra Karen Hernández Guillén,* Víctor A. González Hernández, Estela Sánchez Quintanar**,** Facultad de Química, UNAM |
| **B-56** | **Residual structure of a motif in the intrinsically disordered region of the Escargot protein**. *Teresa Hernández Segura*, Carmen Nina Pastor Colón. IICBA. Universidad Autónoma del Estado de Morelos |
| **B-57** | **Nitric assay evaluation in several samples of plasma and serum.** *Yamil Hernández Urquieta***,** Samuel Treviño Mora, Eduardo Brambila Colombres, Alfonso Díaz Fonseca, Patricia Aguilar Alonso. FCQs, Benemérita Universidad Autónoma de Puebla |
| **B-58** | **Study of osmotic fragility of erythrocytes in Chemistry students UJAT.** *Mayra Alondra Jiménez López***,** Solaina Del Lucero Jiménez López, Yisel Díaz Cáliz, Laura Fabiola Estrada Andrade. Universidad Juárez Autónoma de Tabasco |
| **B-59** | **MicroScale Thermophoresis for the study of (Bio) molecular Interactions.** *Dinorah Leiva***,** Daniel Maturana, Ana Lazic, Philipp Baaske, Stefan Duhr. NanoTemper Technologies INC |
| **B-60** | **The effect of punctual mutations on the stability and aggregation state of human CGI-58 protein.** *Miriam L. Llamas García***,** Gabriela Montero Morán, Samuel Lara González. IPICYT |
| **B-61** | **Energetic bases of multidomain functioning of F1-ATPase β subunit.** *Itzel López González***,** Enrique García Hernández, Guillermo Salcedo Barrientos. Química de Biomacromoléculas. Instituto de Química, UNAM |
| **B-62** | **Characterization of cyclin proteins in *Trichomonas vaginalis*.** *Karla Concepción López Pacheco*, María Imelda López Villaseñor. Instituto de Investigaciones Biomédicas, UNAM |
| **B-63** | **Analyzing protein-ligand interactions using ancestral protein reconstruction.** *Saira Maldonado Puga***,** Jesús A. Banda Vázquez, Alejandro Sosa Peinado. Bioquímica. Facultad de Medicina. UNAM |
| **B-64** | **Easy and Rapid Analysis of Protein Stability by nanoDSF**. *Daniel Maturana***,** Dennis Breitsprecher, Melanie Maschberger, Stefanie Hüttl, Thomas Müller, Stefan Duhr, Philipp Baaske. NanoTemper Technologies INC |
| **B-65** | **Cloning and Expression of ribosomal GTPase LSG1.** *Nancy Gabriela Marcial Bazaldúa, Nuria Sánchez Puig***.** Instituto de Química, UNAM |
| **B-66** | **Paralogous Diversification: Repercussion on Metabolic Fluxes.** *Ximena Martínez de la Escalera Fanjul*, James González, KatjaTummler, Edda Klipp and Alicia González. Instituto de Fisiología Celular, UNAM |
| **B-67** | **Orai1 channel modulates agonist-induced $Ca^{2+}$ release from the endoplasmic reticulum.** *Ericka Martínez Martínez***,** Daniel León Aparicio, José Manuel Galindo, Jesús Valdés, Stefan Feske and Agustín Guerrero Hernández. Departamento de Bioquímica, CINVESTAV Zacatenco |
| **B-68** | **S1P1 receptor differentially associate with Rab proteins upon sphingosine 1 phosphate and FTY720-P**. *Juan Carlos Martínez Morales***,** Ma. Teresa Romero Ávila, J. Adolfo García Sáinz. Instituto de Fisiología Celular. UNAM |
| **B-69** | **Physicochemical characterization of Triosephosphate isomerase in the phylum Proteobacteria.** *Bryan Eduardo Martínez Pastor***,** Sergio Romero Romero, Daniel Alejandro Fernández Velasco. Facultad de Medicina, UNAM |
| **B-70** | **The amoebic oxygen reduction pathway is constitutive and increases under *in vitro* hyperoxia and during liver abscess formation in hamsters.** *Yoalli Martínez Pérez***,** Mario Nequiz Avendaño, Fabiola Santos Ramos, Marisol Méndez Vázquez, Sergio Enríquez Flores, Rusely Encalada Oregón, Erika Luis García, Jaime Marcial Quino, Marco E. Gudiño Zayas, Edith Mendoza Tenorio, Emma Saavedra Lira, Ruy Pérez Tamayo and Alfonso Olivos García. Medicina Experimental. Facultad de Medicina, UNAM |
| **B-71** | **B-dystroglycan is involved in the nuclear envelope organization of differentiated HL-60 cells**. *Ivette Astrid Martínez Vieyra***,** Alan S. Cruz Aguirre, Doris A. Cerecedo Mercado. ENMyH. IPN |
| **B-72** | **Mitochondrial training supercomplexes in *Ustilago Maydis* cultivated with different carbon and nitrogen sources**. *Deyamira Matuz Mares***,** Héctor Vázquez Meza, Juan Pablo Pardo Vázquez. Facultad de Medicina, UNAM |

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| **B-73** | **Electron transport chain and cellular redox state in a thermotolerant yeast.** *Jorge A. Mejía Barajas***,** Omar Ortiz Ávila, José A. Martínez Mora, Salvador Manzo Avalos, Ruth Noriega Cisneros, Christian Cortés Rojo, Alfredo Saavedra Molina. Universidad Michoacana de San Nicolas de Hidalgo |
| **B-74** | **Biochemical Models for the kinesin kinetics**. *Ricardo Antonio Méndez Álvarez***,** José Noé Felipe Herrera Pacheco, Lorena Milflores Flores. Facultad de Ciencias Físico Matemáticas. BUAP |
| **B-75** | **Biochemical characterization of an extracellular cellulase from *Sporothrix schenckii.*** *Alicia del Carmen Hernández Guzmán***,** Alberto Flores Martínez, Patricia Ponce Noyola, Julio César Villagómez Castro. División de Ciencias Naturales y Exactas, Universidad de Guanajuato |
| **B-76** | **Identification of the binding sites of the SBDS and EFL1 proteins to the ribosomal subunit 60S.** *Diana Carolina Montagut Guevara*, Nuria Sánchez Puig. Instituto de Química, UNAM |
| **B-77** | **Study of protein sulfhydration in the *Saccharomyces cerevisiae* proteome.** *Paola Moreno Álvarez***,** Emmanuel Ríos Castro, Francisco Torres Quiroz. Instituto de Fisiología Celular, UNAM |
| **B-78** | **V-ATPase localization before and after acrosome reaction in mammalian sperm.** *Michelle Crisely Munguía Figueroa***,** Claudia Lydia Treviño Santa Cruz, Takuya Nishigaki. Instituto de Biotecnología, UNAM |
| **B-79** | **Role of the cytochrome P450 *TvCyt2*, in the biological associations established by *Trichoderma virens* with phytopatogenic fungi and plants.** *María Daniela Porras Troncoso*, Claudia A. Ramírez Valdespino, Alma Rosa Corrales Escobosa, Kazimierz Wrobel, Vianey G. Olmedo Monfil. Departamento de Biología. DCNE. Universidad de Guanajuato |
| **B-80** | **Hypoxia induces expression of p53 in the white shrimp *Litopenaeus vannamei***. *Dahlia María Núñez Hernández***,** Monserrath Felix Portillo, José Alfredo Martínez Quintana, Alma Beatriz Peregrino Uriarte, Elisa M. Valenzuela Soto, José Guadalupe Soñanez Organis, Gloria Yepiz Plascencia. Centro de Investigación en Alimentación y Desarrollo, A.C. |
| **B-81** | **Interactions between carbon and nitrogen influence the fermentative growth of *Saccharomyces cerevisiae.*** *Ivanna Karina Olivares Marín*, Luis Alberto Madrigal Pérez, Melina Canizal García, Juan Carlos González Hernández, Carlos Regalado González. Universidad Autónoma de Querétaro |
| **B-82** | **Spatio–temporal characterization of $Ca^{2+}$ fluxes induced by acrosomal pH alkalinization in mouse sperm**. *Enrique Ismael Oliver Santiago***,** Alberto Darszon. Universidad Autónoma del Estado de Morelos |
| **B-83** | **Peptide profile characterization and antioxidant properties from hemp seeds (*Cannabis sativa* L).** *Olvera Aguirre Alejandra***,** Ana Cristina Ramírez Anguiano, Fermín Paul Pacheco Moisés, Luna Hilda, Velázquez Juárez Gilberto, Velasco Ramírez Sandra Fabiola. CUCEI. Universidad de Guadalajara |
| **B-84** | **Ferri cuptake regulator protein $GDI_{1248}$ in *Gluconacetobacterdiazotrophicus* Pal 5 strain.** *Belén Lucerito Onofre Ramírez***,** Lucía Soto Urzúa, Luis Javier Martínez Morales, Beatriz Eugenia Baca. Centro de Investigaciones Microbiológicas. ICUAP. BUAP |
| **B-85** | **The U/DAPC is found as different assemblies as showed by BN PAGE after digitonin solubilization.** *Ariadna Jazmín Ortega Lozano***,** Lorena I. Rodríguez Páez, María Isabel Contreras Simuta, Juan Fernando Sanmiguel Minauro. Hospital de Pediatría. Centro Médico Nacional "Siglo XXI". IMSS |
| **B-86** | **Sphingolipid biosynthesis and function in bacteria.** *Jonathan Padilla Gómez***,** Daniela A. García Soriano, Diana X. Sahonero Canavesi, Sebastian Poggio Ghilarducci, Isabel M. López Lara, and Otto Geiger. Centro de Ciencias Genómicas, UNAM |
| **B-87** | **Funtional expression and biochemical and kinetic characterization of thioredoxin (Trx) and glutaredoxin (Grx) proteins from *Taenia solium*.** *Agustin Plancarte Crespo***,** Nava Balderas Gabriela, Maldonado Grande Ziad F, Maldonado Cuña Gerardo. Facultad de Medicina. UNAM |
| **B-88** | **Purification, thermal stability analysis and crystallization trials of the human protein G0S2**. *Edgar Daniel Páez Pérez***,** Rubén Paul Gaytán Colín, Gabriela Montero Morán, Samuel Lara González. IPICYT |
| **B-89** | **Study of Bacillus thuringiensis Cry1Ab and Cry1Ac protoxins interaction with cadherin-like receptor from *Manduca sexta***. *Arlen Peña Cardeña***,** Alejandra Bravo, Mario Soberón, Isabel Gómez. Instituto de Biotecnología, UNAM |
| **B-90** | ***Arabidopsis thaliana* mitochondrial primase helicase: biochemical characterization and protein interaction with organellar polymerases of plants**. *Antolin Peralta Castro***,** Corina E. Díaz Quezada, Luis G. Brieba. LANGEBIO. CINVESTAV. Irapuato |

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| **B-91** | **The Role of *caa$_3$* and *aa$_3$* Oxidases in Supercomplex Formation on the Respiratory Chain of *Bacillus subtilis.*** *Gerardo Ignacio Picón Garrido***,** Emma Berta Gutiérrez Cirlos Madrid. FES Iztacala, UNAM |
| **B-92** | **A new thioredoxin reductase with additional glutathione reductase activity in *Haemonchuscontortus*.** *Agustín Plancarte Crespo***,** Nava Balderas Gabriela, Munguía Xochihua Javier. Facultad de Medicina, UNAM |
| **B-93** | **Partial chemical characterization of zapote fruits (*Diospyrosdigyna and Diospyrosrekoi*) sampled from different regions of western Mexico**. *Ernesto Ramírez Briones*, John Paul Délano Frier, Axel Tissen Favier, Julio A. Massange Sánchez, Norma A. Martínez Gallardo, Julia Zañudo Hernández. CUCBA. Universidad de Guadalajara |
| **B-94** | **MAD2γ in the mitotic check point and its association with taxol resistance in the colorectal cancer cell line HCT116.** *Miguel Ángel Ramírez Otero***,** Alejandro López Saavedra, Luis Alonso Herrera Montalvo. Laboratorio de Carcinogénesis. Instituto Nacional de Cancerología. IIB UNAM |
| **B-95** | **The conversion of a $K^+$-dependent to a $K^+$-independent pyruvate kinase and its gradual improvement in its catalytic efficiency by the contribution of three residues**. *Leticia Ramírez Silva***,** Paul Gómez Coronado, Gloria Hernández Alcántara, Cristina Rodríguez Méndez and Nallely Cabrera. Facultad de Medicina, UNAM |
| **B-96** | **Comparison of production and efficiency of MDMX Polyclonal Antibodies made in Rabbit and Chicken.** *Yolanda Rebolloso Gómez***,** Javier Nuñez and Vanesa Olivares Illana. Instituto de Física, UASLP |
| **B-97** | **Silencing of the hypoxia inducible factor -1α decrease the white spot syndrome virus infection in shrimp muscle**. *Rafael Romero Clark,* José A. Godoy Lugo, Silvia Gómez Jiménez, Luis A. Gámez Alejo, José Gpe. Soñanez Organis. Departamento de Ciencias Químico Biológicas y Agropecuarias, Universidad de Sonora |
| **B-98** | **Bionformatic analysis of genes transcription on conditions of chronological aging**. *Olaf Rosas Galicia***,** Patricia Leany Segundo Ibañez, Lourdes Millán Pérez Peña, Irma Herrera Camacho, Nora Hilda Rosas Murrieta. Centro de Química. ICUAP. BUAP |
| **B-99** | **Transcriptional modification of Fma1, Fma2 y Pca1 genes in the model of chronological aging of *Schizosaccharomyces pombe*.** *Patricia Leany Segundo Ibáñez*, Olaf Rosas Galicia, Lourdes Millán Pérez Peña, Irma Herrera Camacho, Nora Hilda Rosas Murrieta. Centro de Química. ICUAP. BUAP |
| **B-100** | **Betaine Aldehyde Dehydrogenase expression during physiological cardiac hypertrophy in Sprague-Dawley rats**. *Juan Alberto Espinoza Salazar***,** José Arquimides Godoy Lugo, Cesar Jeravy López Jacobo, Jorge Arturo Moroyoqui Yevismea, José Guadalupe Soñanez Organis, Norma Aurora Stephens Camacho, Jesús Alfredo Rosas Rodríguez. Universidad de Sonora |
| **B-101** | **Trypsin activity from larval intestine of *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae).** *Gilberto Ruiz de la Cruz***,** Jorge Ariel Torres Castillo, María Cruz Juárez Aragón. Instituto de Ecología Aplicada. Instituto Tecnológico de Ciudad Victoria |
| **B-102** | **Detection of the oncogenes MDMX, MDM2 and the tumor suppressor RB In Human Blood Sample.** *Karla Lorena Salazar Campos***,** Vanesa Olivares Illana. Laboratorio de interacciones moleculares y cáncer. Universidad Autónoma de San Luis Potosí |
| **B-103** | **The effect of the flavonoid nobiletin in the Akt signalling pathway and VEGF expression in the pharynx cancer *FaDu* cell line.** *Alejandra Ruíz Romero*, Gloria Gutiérrez Venegas. Laboratorio de Bioquímica. División de Estudios de Posgrado e Investigación. Facultad de Odontología, UNAM |
| **B-104** | **Dropout Students of biochemistry and molecular biology**. *Yolanda Saldaña Balmori*, Héctor Javier Delgadillo Gutiérrez. Facultad de Medicina, UNAM |
| **B-105** | **Regulatory role of ADP-PFK1 and FruBPase II on the gluconeogenesis/glycolysis fluxes in *Methanosarcina acetivorans*.** *Michel Geovanni Santiago Martínez***,** Ricardo Jasso Chávez. Departamento de Bioquímica. Instituto Nacional de Cardiología "Ignacio Chávez" |
| **B-106** | **Maintenance of intracellular hypoxia and adequate heat shock response are essential requirements for pathogenicity and virulence of *Entamoeba histolytica***. *Fabiola Santos Ramos***,** Mario Nequiz Avendaño, Jaime Marcial Quino, Sergio Enríquez Flores, Yoalli Martínez Pérez, Erika Pineda Ramírez, Rusely Encalada Oregón, Nancy Guillén, Erika Luis García, Marisol Méndez Vázquez, Marco Gudiño Zayas, Edith Mendoza Tenorio, Emma Saavedra Lira, Ruy Pérez Tamayo and Alfonso Olivos García. Facultad de Medicina, UNAM |

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| **B-107** | **Structural and functional characterization of an amaranth seed LEA Protein**. *Alma Leticia Saucedo Yáñez*, Eric Edmundo Hernández Domínguez, Daniel Alejandro Fernández Velasco, Ana Paulina Barba de la Rosa. División de Biología Molecular, IPICYT |
| **B-108** | **Small angle X ray Scattering (SAXS) studies of the low resolution structure of the ribosomal GTPAseEFL1, the SBDS protein and their complex**. *Dritan Siliqi*, Davide Altamura, Abril Gijsbers, Eugenio de la Mora, Cinzia Giannini, Teresa Sibillano, Michele Saviano, Nuria Sánchez Puig. Istituto di Cristallografia. Consiglio Nazionale delle Ricerche (CNR) |
| **B-109** | **The size of gold nanoparticles affects the antimicrobial activity.** José de Jesús Olivares Trejo*, José Domingo Trujillo Casarreal* and Norma Velázquez Guadarrama**.** Posgrado en Ciencias Genómicas, Universidad Autónoma de la Ciudad de México |
| **B-110** | **Curcumin inhibits the thioredoxin-glutathione reductase (TGR) from larva *Taenia crassiceps* (cysticerci)?** *Rubén Solís Cruz***,** Alberto Guevara Flores, José de Jesús Martínez González, Juan Luis Rendón Gómez, Irene Patricia del Arenal Mena. Facultad de Medicina. UNAM |
| **B-111** | **Peroxisome/mitochondrial dynamics and development of *Podospora anserina* in a *fis1* deletion strain.** *Harumi Takano Rojas***,** Jorge Luis Castillo Canizáles, Leonardo Peraza Reyes. Instituto Potosino de Investigación Científica y Tecnológica, A.C., UNAM |
| **B-112** | **Purification of a type lectin protein from pericarp *Opuntia ficus-indica* that shown agglutination and cytotoxic effect.** *Lizbeth Tehuitzin Acevedo***,** Juana Pileño García, Carlos César Patiño Morales, Javier Jiménez Hernández, Eneas Alejandro Chavelas Adame. Unidad Académica de Ciencias Químico Biológicas. Universidad Autónoma de Guerrero |
| **B-113** | **Probing the phosphoryl-group transfer routes in the ArcB dimer**. *Juan Luis Terán Melo***,** Adrian Fernando Alvarez, Dimitris Georgellis. Departamento de Genética Molecular, Instituto de Fisiología Celular. UNAM |
| **B-114** | **Characterization and inhibition of Polyphenol oxidase fromHass avocado (*Persea americana*).** *Zaira Ileana Tobías Juárez***,** Lorena Xolalpa Cueva Hugo Nájera Peña. Universidad Autónoma Metropolitana. Unidad Cuajimalpa |
| **B-115** | **Claudin-6, -7 and -9 transfected AGS cells induce Hsp-27, -40, -70 and -90 protein expression**. *Priscila J. Torres Granados***,** María de la Luz Pérez Uscanga, Erika P. Rendón Huerta, Luis F. Montaño Estrada. Facultad de Medicina, UNAM |
| **B-116** | **Evaluation of cytotoxic, antioxidant and anti-inflammatory activity of essential oil and terpenes of *Saturejamacrostema***. *Rafael Torres Martínez***,** Alejandra Hernández García, Alfredo Saavedra Molina, Joel Edmundo López Meza, Alejandra Ochoa Zarzosa and Rafael Salgado Garciglia. Instituto de Investigaciones Químico Biológicas. Universidad Michoacana de San Nicolás de Hidalgo |
| **B-117** | **Construction of variants of Cyt1A toxin from the *Bacillus thuringiensis* to migrate their toxicity to lepidopteran insects**. *Mary Carmen Torres Quintero***,** Mario Soberón, Alejandra Bravo. Instituto de Biotecnología, UNAM |
| **B-118** | **IL-8 secreted by AGS cells modify transepithelial electrical resistance and leukocyte adhesion on HUVECs**. *Jahaziel Caleb Tovar Mota***,** José Francisco Gallardo, Martín Gallardo, Luis Felipe Montaño Estrada, Erika Patricia Rendón Huerta. Facultad de Medicina, UNAM |
| **B-119** | **Dispensability of the [4Fe-4S] cluster in novel homologues of adenine glycosylase MutY**. *Carlos H. Trasviña Arenas***,** Laura M. Lopez Castillo, Eugenia Sánchez Sandoval, Corina Díaz Quezada and Luis G. Brieba. LANGEBIO. CINVESTAV. Irapuato |
| **B-120** | **Revealing the structural and energetics basis of ABL tyrosine kinase inhibition by target-directed drugs.** *Mario Trejo Pérez***,** Roberto Cruz Castañeda, Ignacio Regla, Enrique García Hernández, Axel Luviano. Instituto de Química, UNAM |
| **B-121** | **Metabolomic study of *Lasiodiplodia theobromae***. *Carla Uranga Solis***,** Joris Beld, Anthony Mrse, Ivan Córdova Guerrero, Michael Burkart, Rufina Hernández Martínez. Centro de Investigación Científica y de Educación Superior de Ensenada |
| **B-122** | **Study of the NAC-mediated interactions between cytosolic ribosomes and Sam37 during mitochondrial protein import**. *Miguel Gandi Valdés Dávila*, Ponce Rojas José Carlos, Jaimes Miranda Fabiola & Funes Soledad. Instituto de Fisiología Celular, UNAM |

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| **B-123** | **Evaluation of the Antimicrobial Activity of Components of the Venom of the Snake *Bothrops ammodytoides***. *Alexis J. Rodríguez Solís*, Eduardo Vargas Salgado, Herlinda Clement Carretero, Gerardo A. Corzo Burguete, Adolfo R. De Roodt, Elba C. Villegas Villarreal. Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos |
| **B-124** | **Mitochondrial training supercomplexes in *Ustilago maydis* cultivated with different carbon and nitrogen sources**. *Deyamira Matuz Mares*, Héctor Vázquez Meza**,** Juan Pablo Pardo Vázquez. Departamento de Bioquímica. Facultad de Medicina, UNAM |
| **B-125** | ***In vitro* import of subunit Atp6 of the ATP synthase into mitochondria of the chlorophyce an alga *Polytomella* sp.** *Félix Vega de Luna***,** Miriam Vázquez Acevedo, Diego González Halphen. Instituto de Fisiología Celular, UNAM |
| **B-126** | **CatSper, a specific cation channel of sperm is involved in sperm chemotaxis in sea urchin**. *Martín Velázquez Pérez***,** Héctor Ramírez, Adán Guerrero, Alberto Darszon, Carmen Beltrán. Facultad de Ciencias Biológicas. Universidad Autónoma del Estado de Morelos |
| **B-127** | **UMAG_02301 gene encoding a transcription factor is involved in the pathogenesis of *Ustilago maydis* in maize**. *John Martin Velez Haro,* Lorenzo Guevara Olvera, and José Ruiz Herrera. Instituto Tecnológico de Celaya. CINVESTAV Unidad Irapuato |
| **B-128** | **Purification and characterization of digestive trypsin-like and chymotrypsin-like proteases in the larger grain borer *Prostephanus truncatus* (Horn).** *Viviana Villalobos Murillo***,** Jimena Meneses Plascencia, Alejandro Blanco Labra. CINVESTAV Unidad Irapuato |
| **B-129** | **Easy Expression and Purification of Histidine-tagged Bacteriophage T7 DNA Polymerase in a 1:1 complex with Thioredoxin**. *Jesús G. Zendejas Sánchez***,** Corina E. Díaz Quezada Luis G. Brieba de Castro LANGEBIO. CINVESTAV Irapuato |
| **B-130** | **Physicochemical characterization of monomeric proteins with (β/α)8-barrel topology: looking for unfolding reversibility**. *Susana Vázquez Torres*, Sergio Romero Romero. Daniel Alejandro Fernández Velasco. Facultad de Medicina UNAM |
| **B-131** | **Characterization of thehydrophobic subunits of theperipheral arm of the ATP synthase from the colorless alga *Polytomella* sp.** *Lorenzo Sánchez Vásquez*, Miriam Vázquez Acevedo, Francisco Javier de la Mora Bravo, Georges Dreyfus, Diego González Halphen. Instituto de Fisiología Celular, UNAM |
| **B-132** | **Cloning, optimization, expression and purification of the α-glucosidase *Ruminococcus obeum*, to be used as a molecular target in the discovery of new antidiabetic drugs.** *María Isabel Velázquez López* and Martin González Andrade. Facultad de Medicina, UNAM |
| **B-133** | **Effects of Cu (II) and Zn (II) in HgD crystalline aggregation over time**. *Arline Fernandez Silva*, Lina Rivillas Acevedo, Liliana Quintanar Vera, Jonathan King, Carlos Amero Tello. Centro de Inv. Químicas. UAEM |
| **B-134** | **Effect of human alfa crystallin peptides in the human γD-crystallin aggregation**. *Hugo A. Gómez Uribe*, Carlos Amero, Lina Rivillas. Laboratorio de Bioquímica y Resonancia Magnética Nuclear, Centro de Investigaciones Químicas, UAEM |
| **B-135** | **Spectroscopic study of Cu (II) binding to light chain 6aJL2-R24G and its effect on amyloid fiber formation**. *Angel E. Peláez Aguilar*, Carlos Amero, Lina Rivillas Acevedo. Centro de Investigación en Dinámica Celular, Instituto de Investigación en Ciencias Básicas y Aplicadas, UAEM |
| **B-136** | **Kinetic characterization of paralogous enzymes in *Saccharomyces cerevisiae* Bat1 and Bat2: branched aminoacid aminotransferases.** *Beatriz Aguirre López*, José Carlos Campero Basaldúa, Eréndira Rojas, Dariel Márquez, Maritrini Colón, Lina Riego, Javier Montalvo and Alicia Gonzalez. Instituto de Fisiología Celular, UNAM |
| **B-137** | **Purification and characterization of Alt1 and Alt2 of *Saccharomyces cerevisiae*: Study of structural divergence.** Rojas Ortega Eréndira, *Aguirre López Beatriz,* Granados Avalos Estefany, González Andrade Martín, Guerrero Miguel Ángel, Reyes Vivas Horacio, Tuena Sangri Marietta, González Manjarrez Alicia. Instituto de Fisiología Celular, UNAM |
| **B-138** | **Identification of an-arginine endoplasmic reticulum (ER) retention motif and induction of oocyte maturation by a G-protein activated potassium channel.** *Claudia Iveth Rangel García*, Díaz Bello Beatriz, Salvador Carolina, Escobar Laura. Facultad de Medicina, UNAM |

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| **B-139** | **Regulation of the hyperpolarization-activated cyclic nucleotide-gated HCN channels by the serum and glucocorticoid-inducible kinase 1 (SGK1).** *Erika Gutiérrez Vásquez*, Teresa Padilla Flores, Carolina Salvador Hernández, Laura I Escobar Pérez. Facultad de Medicina, UNAM |
| **B-140** | **Cloning, Expression and Kinetics of the Enzyme Amino Aldehyde Dehydrogenase PA5312 of *Pseudomonas aeruginosa.*** *Yudy Vanesa Cardona Cardona*, Javier Carrillo Campos, Carlos Mújica Jiménez, Rosario A. Muñoz Clares. Facultad de Química, UNAM |

## OXYGEN REACTIVE SPECIES

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| **OR-1** | **Cisplatin induced damage in LLC-PK1 cells: cytoprotective effect of Morin without interfering with cisplatin toxicity on HTB-4 cells.** *Claudia Bello Álvarez***,** Ángela Patricia Moreno Londoño, José Pedraza Chaverri. Facultad de Química, UNAM |
| **OR-2** | **Has the heterologous expression of *Debaryomyces hansenii* catalase T any effect in *Saccharomyces cerevisiae* chronological life span?** *Román Alfonso Castillo Díaz*, Ileana de la Fuente Colmenares, Luisa Alba Lois, Víctor Valdés López, Viviana Escobar Sánchez, Claudia Segal Kischinevzky. Facultad de Ciencias, UNAM |
| **OR-3** | **Measurement of markers lipid peroxidation (MDA and 4-HDA) in blood serum in response to stressful conditions in students**. *Iván Cesar Arteaga*, Leticia García Albarrán, Raúl Ávila Sosa Patricia Aguilar Alonso. Facultad de Ciencias, BUAP |
| **OR-4** | **Evaluation of tumour necrosis factor alpha (TNF-alpha) and nitric oxide gene expression in liver's rats fed with Spirulina platensis and subjeted to endotoxic shock**. *Luis Carlos Comparan Moreno*, Sandra Fabiola Velasco Ramírez, Ramírez Anguiano Ana Cristina, Cortes Romero Celso, Pacheco Moisés Fermín Paul, González Ortiz Luis Javier, Sánchez Peña María Judith, Bitzer Quintero Oscar Kurt, Rosales Rivera Lizet Yadira, Hilda Luna Zaizar, Gilberto Velázquez Juárez. Centro Universitario de Ciencias Exactas e Ingenierías, Universidad de Guadalajara |
| **OR-5** | **Revealing the role of selective mitochondrial autophagy in yeast longevity**. *Francisco Daniel Dávila Alemán,* Soledad Funes, Alfredo Herrera Estrella, Alexander de Luna. LANGEBIO, CINVESTAV Irapuato |
| **OR-6** | **Expression analysis of *Debaryomyces hansenii catalase* T in *Saccharomyces cerevisiae* as host.** *Ileana de la Fuente Colmenares*, Román Castillo Díaz, Viviana Escobar Sánchez, Luisa Alba Lois, Víctor Valdés López and Claudia Segal Kischinevzky. Facultad de Ciencias, UNAM |
| **OR-7** | **Induction of Class III peroxidase secretion from maize scutellum in early postgermination in relation with the season of the year**. *David Manuel Díaz Pontones*, Claudia Ponce Sánchez, José Isaac Corona Carrillo. Departamento de Ciencias de la salud, Universidad Autónoma Metropolitana Iztapalapa |
| **OR-8** | **Relationship between enzymatic and non-enzymatic antioxidants with muscle mass loss in the perimenopausal period.** *Oswaldo Daniel García Anaya*, Martha Asunción Sánchez Rodríguez, Ixel Venecia González Herrera, Elsa Correa Muñoz, Víctor Manuel Mendoza Núñez. Facultad de Estudios Superiores Zaragoza, UNAM |
| **OR-9** | **Iron mediated-ISC system of oxidative damage and mitochondrial dysfunction in *Saccharomyces cerevisiae***. *Mauricio Gómez Gallardo*, Luis Alberto Sánchez Briones, Alma Laura Díaz Pérez, Christian Cortes Rojo and Jesús Campos García. Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo. |
| **OR-10** | **SRX1 encodes a sulfiredoxin required to support the oxidative stress in *Candida glabrata.*** *Gutiérrez Escobedo Ma. Guadalupe*, Nájera Martínez Minerva, Castaño Navarro Irene Beatriz and De Las Peñas Nava Alejandro.IPICYT |
| **OR-11** | **Thioredoxin-glutathione reductase is an ancestral enzyme in the Animal Kingdom. An evolutionary proposal about its importance in the antioxidant systems in animals**. *José de Jesús Martínez González,* Zurisadai Miguel Muñoz González, Alberto Guevara Flores, Juan Luis Rendón Gómez, Patricia del Arenal Mena. Facultad de Medicina, UNAM |

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| **OR-12** | **Effect of aged garlic extract on the level of reactive oxygen species and phosphorylation of AMPK in muscle tissue of diabetic rats.** *Juan Miguel Mendoza Bello*, Martha Isela Barragán Bonilla, Gloria Fernández Tilapa, Mónica Espinoza Rojo. Laboratorio de Investigación en Biología Molecular y Genómica, Universidad Autónoma de Guerrero |
| **OR-13** | **Preliminary phytochemical analysis of Guácima (*Guazuma ulmifolia* Lam.) steams, seeds and leafs.** *Diana Isabel Montoya Salazar*, Paulina Elizabeth Torres Galindo, Alejandro Moreno López, Erika Guadalupe Medina Vega, Luis Carlos Comparan Moreno, Vania Montserrat Salazar Camarillo, Daniel Rogelio Flores Mendoza, José de Jesús Luna Díaz, Hilda Luna Zaizar, Ana Cristina Ramírez Anguiano, Gilberto Velázquez Juárez, Sandra Fabiola Velasco Ramírez. Centro Universitario de Ciencias Exactas e Ingenierías, Universidad de Guadalajara |
| **OR-14** | **Isoliquiritigenin attenuates cisplatin induced proximal tubule cell death.** *Ángela Patricia Moreno Londoño*, Claudia Bello Álvarez, José Pedraza Chaverri. Facultad de Química, UNAM |
| **OR-15** | **Computational Study of Chemical Reactivity in Antioxidant Systems.** *Zurizadai Miguel Muñoz González*, Felipe Aparicio Platas. Universidad Autónoma Metropolitana |
| **OR-16** | **Redox balance in the interaction of *Trichoderma Arabidopsis*, role of TaTrx2 thioredoxin**. *Alma María de Jesús Ortega Olmos,* Paulina Guzmán Guzmán, Luis Cárdenas Torres, Vianey Graciela Olmedo Monfil. Universidad de Guanajuato |
| **OR-17** | **Functional Nox4 is required for development of progressive motility in spermatozoa of guinea pig.** *Ortiz García Cesar Ismael*, Roa Espitia AL, Hernández González Enrique O. CINVESTAV Zacatenco |
| **OR-18** | **DEND-CUR-(G2)-OH, a new synthetic compound derivate of curcumin, turns off the overexpression of Nrf2 and causes cellular death by production of reactive oxygen species and autophagic activation on C6 glioma cells.** *Alma Teresa Pérez González*, Martín Landeros Gálvez, Mario Israel Pérez Padrón, Patricia Guevara Salazar, Fernando Belmont Bernal, Patricia Guadarrama Acosta, Irma Gabriela González Herrera. Instituto Nacional de Neurología y Neurocirugía |
| **OR-19** | **Effect of Moringa oleifera on kinetic parameters of diabetic liver catalase**. *Karla Giselle Reyes Moreno,* Mónica A Valdez Solana, Jorge A Sosa Gutiérrez, Ulrik H Pedroza Dávila, María A Sánchez Muñoz, Edson D. Mata Guerra, Erick Sierra Campos. Universidad Juárez del Estado de Durango |
| **OR-20** | **Time-dependent and tissue-specific changes in redox state and oxidative stress during fasting.** *Pedro Rojas Morales*, Angélica Saraí Jiménez Osorio, Edilia Tapia, Laura Gabriela Sánchez Lozada, Diana Barrera Oviedo, Omar Noel Medina Campos, José Pedraza Chaverri. Facultad de Química, UNAM |
| **OR-21** | **Hormone therapy decreases oxidative stress in postmenopausal women with metabolic syndrome.** *Ana Karen Ruiz Rodríguez*, Martha A. Sánchez Rodríguez, Mariano Zacarías Flores, Víctor Manuel Mendoza Núñez. Facultad de Estudios Superiores Zaragoza, UNAM |
| **OR-22** | **Analysis of the antioxidant activity of the hexanic extract of *Eryngium carlinae, in vitro* and in *Saccharomyces cerevisiae* as biological model**. *Donovan J Peña Montes*, Jorge A Mejía Barajas, Mónica Clemente Guerrero, Salvador Manzo Avalos, Rafael Salgado Garciglia, Christian Cortés Rojo, Ruth Noriega Cisneros, Alfredo Saavedra Molina. Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo |
| **OR-23** | **Antioxidant activity of bioactive compounds present in the organic waste of the insect *Ulomoides dermestoides***. *Vania Montserrat Salazar Camarillo*, Daniel Rogelio Flores Mendoza, José de Jesús LunaDíaz, Ana Cristina RamírezAnguiano, Gilberto Velázquez Juárez, José Luis Navarrete Heredia, Sandra Fabiola Velasco Ramírez. Chemistry department, Universidad de Guadalajara |
| **OR-24** | **Resveratrol effect in cardiomyocytes during aging process in rats**. *Jesús Emiliano Toscano Jiménez*, Rubén Ávalos López, Addí Rhode Navarro Cruz, Obdulia Vera López, Leticia García Albarrán Alfonso, Daniel Diaz Fonseca, Samuel Treviño Mora, Patricia Aguilar Alonso. Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla |

# *Tuesday*

**8:30 – 10:30 Plenary Lectures**

8:30 – 9:30 DNA topology and the regulation of transcription
**David Levens**
National Cancer Institute, Bethesda, Maryland, USA

Chair: *Mario Zurita*
Instituto de Biotecnología, UNAM

9:30 – 10:30 Neural coding of subjective sensory experience and uncertainty of perceptual decisions
**Ranulfo Romo**
El Colegio Nacional & Instituto de Fisiología Celular, UNAM

Chair: *Miguel Lara Flores*
Instituto de Biología, UNAM

10:30 – 11:00 Coffee break
Foyer San Marcos Room

**Concurrent Sessions**
Tuesday November 8, 2016

| 11:00 – 13:30 | **4. Microbiology & Viruses**<br><br>**San Marcos One Room** | **5. Basic Biochemistry**<br><br>**San Marcos Two Room** | **6. Genetic, Genetic Regulation & Epigenetic**<br>**San Marcos Three Room** |
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| | Chair: *Gonzalo Izaguirre* University of Illinois at Chicago | Chair: *Guadalupe Espín* Instituto de Biotecnologia, UNAM | Chair: *Juan Miranda Rios* Instituto de Investigaciones Biomédicas. UNAM |
| 11:00-11:20 | **Characterization of the antimicrobial activity of a recombinant 110-kDa membrane protein produced by *Pediococcus acidilactici* ATCC 8042.** *Paola Barbosa González*, Israel García Cano Amelia Farrés. Facultad de Química, UNAM | **Pharmacological and Functional Characterization of the Endogenous LPA1/3 Receptors in A594 Cells.** *Gabriel Carmona Rosas*, M. Teresa Romero Ávila, Marco Alfonzo Méndez, J. Adolfo García Sáinz. Instituto de Fisiología Celular, UNAM | **The CDK8 module of Mediator controls vegetative phase change in *Arabidopsis thaliana.*** *Manuel Buendía Monreal*, Stewart Gillmor. LANGEBIO. CINVESTAV Irapuato |
| 11:20-11:40 | **Effect of immunomodulatory molecules on the gene expression of *Staphylococcusaureus* virulence factors: implications during bacterial internalization into bovine mammary epithelial cells.** *Minerva Frutis Murillo*, Marcelo Alejandro Sandoval Carrillo, Nayeli Alva Murillo, Alejandra Ochoa Zarzoza, Joel Edmundo López Meza. Facultad de Medicina Veterinaria y Zootecnia, Universidad Michoacana de San Nicolás de Hidalgo | **The crosstalk between several posttranslational modifications controled the transcriptional activity of deltalactoferrin.** *Adelma Escobar Ramírez*, Anne Sophie Vercoutter Edouart, Isabelle Huvent, Marlene Mortuaire, Tony Lefebvre and Annick Pierce. CNRS-Université des Sciences et Technologies de Lille, IFR | **Identification of physiological and molecular responses associated to abscisic acid under hydric stress in a new model of plant *Marchantia polymorpha.*** *Damaris Godínez Vidal*, Alejandra Alicia Covarrubias Robles, and José Luis Reyes Taboada. Instituto de Biotecnología, UNAM |
| 11:40-12:00 | **Cockatiel (*Nymphicus hollandicus*) gut microbiomes, bacterial inhabitants of a worldwide distributed pet.** *Apolinar M Hernández Gómez*, Luis David Alcaráz, Mariana Peimbert Universidad Autónoma Metropolitana. Unidad Cuajimalpa | **Inherent conformational Flexibility of F1-ATPase α-Subunit.** *Enrique García Hernández*, Otto Hahn Herrera, Guillermo Salcedo Xavier Barril. Instituto de Química, UNAM | **Evaluation of miR-196a i the development and progression of cervical cancer.** *Yahir Alberto Loissell Baltazar*, Oliver Millán Catalán, Abraham Pedroza Torres, Jorge Francisco Cerna Cortés, Carlos PérezPlasencia. Instituto Nacional de Cancerología |
| 12:00-12:20 | **Phenotypic plasticity of *Bacillus* isolates from Coahuila, Mexico.** *Hurtado Barbosa Enrique*, Diana Fabiola Díaz Jiménez, Diana Guadalupe Tapia García and Gabriela Olmedo Álvarez. CINVESTAV Irapuato | **Single molecule measurements of kinesin dynamics and DNAprotein interactions.** *Braulio Gutiérrez Medina*. IPICYT | **Characterization of *cis*-elements that negatively regulate transcription of EPA genes through silencing proteins of *Candida Glabrata.*** *Eunice López Fuentes*, Irene Castaño. IPICYT |

| | **4 Microbiology & Viruses**<br><br>**San Marcos One Room** | ***5 Basic Biochemistry***<br><br>***San Marcos Two Room*** | **6 Genetic, Genetic Regulation & Epigenetic**<br>**San Marcos Three Room** |
|---|---|---|---|
| | Chair: *Gonzalo Izaguirre*<br>University of Illinois at Chicago | Chair: *Guadalupe Espín*<br>Instituto de Biotecnologia, UNAM | Chair: *Juan Miranda Rios*<br>Instituto de Investigaciones Biomédicas. UNAM |
| 12:20-12:40 | **Proprotein Convertase Selectivity in the Activation of the Human Papilloma Virus.** *Gonzalo Izaguirre*. University of Illinois at Chicago. | ***In vivo* detection of compounds from plants by mass spectrometry using low-temperature plasma (LTP) ionization.** *Sandra Martínez Jarquín*, Humberto Herrera Ubaldo, Stefan De Folter, Robert Winkler. CINVESTAV Irapuato | **Regulatory divergence in paralogous genes *ALT1* and *ALT2*: Rtg3-Nrg1-Gln3 negative hybrid complex regulator modulates transcriptional repression** *Dariel Márquez*, Hugo Hernández, James González, José Carlos Campero Basaldúa and Alicia González. Instituto de Fisiología Celular, UNAM |
| 12:40-13:00 | **Influence of resveratrol in the chronological life span of *Saccharomyces cerevisiae.*** *Luis Alberto Madrigal Pérez*, Ivanna Karina Olivares Marín, Lillian Camacho Torres, Juan Carlos González Hernández, Gerardo M. Nava and Minerva Ramos Gómez Instituto Tecnológico Superior de Ciudad Hidalgo | **Hsp90α and Hsp90β are differentially expressed during the anti-proliferative and anti-apoptotic effect of the 17-DMAG inhibitor and thus dictate the treatment resistance of prostate cancer cell lines.** *Javier Octavio Mejía Hernández*, José Eduardo Pérez Aquino, Luis Alonso Herrera Montalvo, Carlo César Cortés González. Unidad de Investigación Biomédica en Cáncer del IIB-UNAM | **Selection & validation of housekeeping genes and differential expression of stress related genes in *Debaryomyces hansenii* subjected to osmotic stress.** *Diana Julieta Martínez Pérez*, Viviana Escobar Sánchez, Víctor Valdés López, Luisa Alba Lois and Claudia Segal Kischinevzky. Facultad de Ciencias,UNAM |
| 13:00-13:20 | **The microbiome of CAM plants: diversity and functional strategies in drylands.** *Laila Pamela Partida Martínez*, Damaris Desgarennes, Citlali Fonseca García, Devin Coleman Derr, Víctor M. Flores Núñez, David A. Camarena Pozos, Etzel Garrido, Axel Visel, Susannah G. Tringe. Departamento de Ingeniería Genética, CINVESTAV Irapuato | **Light chain amyloidosis: from immunoglobulin unfolding to amyloid-like protofibrils.** *Gilberto Valdés García*, César Millán Pacheco, Nina Pastor. Centro de Investigación en Dinámica Celular, Universidad Autónoma del Estado de Morelos | **Biochemical characterization of the putative protein Rad51 from *Milnesium tardigradum*** *Mariana Lucía Martínez Rodríguez,* Guadalupe Ortega Pierres, Rosa María Bermúdez Cruz. CINVESTAV Zacatenco |

13:30 – 15:30 Lunch

**Plenary Session 4**
Tuesday November 8, 2016
15:30 – 18:00 San Marcos Room I

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**New approaches on the study of bacteria**

Chair: *Gloria Soberón*
Instituto de Investigaciones Biomédicas, UNAM

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15:30 – 16:00 Insect endosymbionts: bacteria or organelles?
**Esperanza Martínez Romero**, Tabita Shamayim Ramírez Puebla, Mónica Rosenblueth, Tania Rosas Pérez, Julio Martínez Romero, Arturo Vera Ponce León, Luis Servín Garcidueñas, Ernesto Ormeño Orrillo
Center for Genomic Sciences, UNAM

16:00 – 16:30 Lessons on genomic plasticity from bacteria from microbial communities
**Gabriela Olmedo Álvarez**
Ingeniería Genética, CINVESTAV Unidad Irapuato

16:30 – 17:00 Different coats for different challenges: adjusting the bacterial membrane to distinct environments
**Otto Geiger**, Diana X. Sahonero Canavesi, Lourdes Martínez Aguilar, Christian Sohlenkamp, and Isabel M. López Lara
Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México

17:00 – 17:30 Evolution of mitochondria: when, where and from which bacteria?
**Mauro Degli Esposti**
Italian Institute of Technology, Genoa, Italy

17:30 – 18:00 Are essential genes always part of the core genome?
**Gloria Soberón Chávez**, Enrique Martínez Carranza, Luis Servín González, Hugo Barajas, Luis David Alcaráz
Instituto de Investigaciones Biomédicas, UNAM

**Plenary Session 5**
Tuesday November 8, 2016
15:30 – 18:00 San Marcos Room II

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**Biochemistry and Plant Molecular Biology**

"*In Memoriam* Dr. Federico Sánchez Rodríguez"

Chair: *Jean Phillipe Vielle*
LANGEBIO CINVESTAV Irapuato

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15:30 – 16:00 An autophagy-related kinase is essential for *Phaseolus vulgaris* symbiosis with both *Rhizobium* and arbuscular mycorrhizal fungi
Georgina Estrada Navarrete, Neftaly Cruz Mireles, Ramiro Lascano, Xóchitl AlvaradoAffantranger, Alejandra Hernández, Aarón Barraza, Juan E. Olivares, Manoj Kumar Arthikala, Luis Cárdenas, **Carmen Quinto** and Federico Sánchez
Biología Molecular de Plantas. Instituto de Biotecnología, UNAM

16:00 – 16:30 Origin and genome shaping of common bean, from uncovering its closest sister species to its domestication in America
**Alfredo Herrera Estrella**, Martha Rendón Anaya, Josaphat M. Montero Vargas, Soledad Saburido Álvarez, Anna Vlasova, Salvador Capella Gutierrez, José Juan Ordaz Ortiz, Luis Delaye Arredondo, Toni Gabaldón, Paul Gepts, Robert Winkler, Roderic Guigó, Alfonso Delgado Salinas
LANGEBIO CINVESTAV Irapuato

16:30 – 17:00 Multidisciplinary Research Needed for Sustainability of Global Phosphorus
**C.P. Vance**, J.J. Elser, W.R. Scheible
Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN

17:00 – 17:30 Orchid diversity in México
**Salazar Gerardo A**, Hágsater Eric, Solano Gómez Rodolfo, Huerta Espinoza Héctor
Departamento de Botánica, Instituto de Biología, UNAM

**Plenary Session 6**
Tuesday November 8, 2016
15:30 – 18:00 San Marcos Room III

**Signal Transduction**

Chair: *Roberto Sánchez Olea*
Instituto de Física, UASLP

15:30 – 16:00 Gpn1 and Gpn3, a shared adventure
**Roberto Sánchez Olea** and Mónica R. Calera
Instituto de Física, Universidad Autónoma de San Luis Potosí

16:00 – 16:30 Regulation of the innate immune response of the intestinal epithelial cells by PI3K/Akt signaling during colitis
**Porfirio Nava**, Antonio Hernández Trejo, Itzel Gutierrez Martinez, Aurora Candelario Martínez, Carolina Serrano, Omar E. Fernández Vargas, Michael Schnoor and Nicolás Villegas Sepulveda
Department of Physiology, Biophysics, and Neurosciences. CINVESTAV. Zacatenco

16:30 – 17:00 Functional interaction of hypoxia-inducible factors and autophagy mediates drug resistance in colon cancer cells.
**Martha Robles Flores**, Abril Saint Martin and M. Cristina Castañeda Patlán
Department of Biochemistry, Faculty of Medicine, UNAM

17:00 – 17:30 EMT and migration in ovarian carcinoma cells are regulated by UTP and adenosine.
**Francisco Gabriel Vázquez Cuevas**, Angélica Sofía Martínez Ramírez and Mauricio Díaz Muñoz
Departamento de Neurobiología Celular y Molecular. Instituto de Neurobiología, UNAM

17:30 – 18:00 G-proteins regulate mitotic spindle formation
**Guadalupe Reyes Cruz**, Misael Neri Dionisio Vicuña, Tania Yareli Gutiérrez López and José Vázquez Prado
Departments of Cell Biology. CINVESTAV. Zacatenco

18:00 – 20:00 Poster Session 2

BT BIOTECHNOLOGY
M MEDICINE, HEALTH AND NUTRITION

20:00 Bussiness Session – SMB

## BIOTECHNOLOGY

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| **BT-1** | **Industrial interest exoenzymes production by acidometalophilic bacteria isolated from oxidized mine tailings**. *José Luis Aguirre Noyola,* Gustavo Cuaxinque Flores, José Daniel Chávez González, Oscar Talavera Mendoza, Yanet Romero Ramírez, Jeiry Toribio Jiménez. Universidad Autónoma de Guerrero |
| **BT-2** | **Interaction proteomics of Op14-3-3mu protein of *Opuntia ficusindica*.** *Ana Paulina Barba de la Rosa*, Eric Edmundo Hernández Domínguez, Abraham Escobedo Moratilla, Alberto Barrera Pacheco. Biomedicina, Instituto Potosino de Investigación Científica y Tecnológica A.C. |
| **BT-3** | **Study of expression of *pvdS* and *csbC* genes in *Azotobacter vinelandii*.** *Thalía Barrientos Millán*, Liliana López Pliego, Miguel Castañeda Lucio. Centro de Investigaciones en Ciencias Microbiológicas. Benemérita Universidad Autónoma de Puebla |
| **BT-4** | **Partial characterization of antimicrobial substances produced by *Lactobacillus paracasei* KSI.** *Juan Carlos Benítez Serrano,* Laura Martínez Pérez, Azarel Ruíz Román, Ricardo Carrasco Torres, Nora Hilda Rosas Murrieta, Eduardo Miguel Brambila Colombres, Rigoberto Hernández Castro, Mónica Rosales Pérez, Patricia Aguilar Alonso. Benemérita Universidad Autónoma de Puebla |
| **BT-5** | **Characterization of mutant $PilR_{D53N}$ in the two component system PilS-PilR of *Geobacter sulfur reducens* and its relationship with the electron transfer.** *Xadeni Burgos Gámez,* Alberto Hernández Eligio y Katy Juárez López. Instituto de Biotecnología, UNAM |
| **BT-6** | **Micropropagation of *Mammillaria rhodantha* in temporary immersion system.** *Juan Carlos Camargo Tavares,* Lisset Herrera Isidrón, Héctor Gordon Núñez Palenius. Unidad Politécnica Interdisciplinaria de Ingeniería Campus Guanajuato (UPIIG) |
| **BT-7** | **Production and purification of the leucin-aminopeptidase yspII produced in the yeast *Pichia pastoris.*** *Lorena Cardona Fuentes,* Nora Rosas Murrieta, Lourdes Millán Pérez Peña, Irma Herrera Camacho. Centro de Química ICUAP. BUAP |
| **BT-8** | **Interaction between Bcl-2 and Bax proteins.** *Caro Gómez Luis A,* Vique Sánchez José L, Mixcoha Hernández Edgar, Rosas Trigueros Jorge L, Benítez Cardoza Claudia G, Zamorano Carrillo Absalom. Laboratorio de Bioquímica y Biofísica Computacional, ENMH, Instituto Politécnico Nacional |
| **BT-9** | **Central nervous systems effects of nanoparticles administered orally.** *Anahi Chavarria Krauser,* Claudia Meza López y Olguín, Esperanza García, Gerardo Andrés Vega Rosas, Sandra Romero Alvarado, Fanny García Cruz. Facultad de Medicina, UNAM |
| **BT-10** | **Determination of growth kinetic parameters of the plant pathogen *Ralstonia solanacearum*.** *María Inés Chávez Bejar,* José Luis Mendoza Porcayo, Jesús Hernández Romano. Universidad Politécnica del Estado de Morelos |
| **BT-11** | **Heavy metal-resistance profile of diazotrophic bacteria isolated from root of *Acacia farnesiana*(L) Willd that grow in acidic mine tailings.** *José Daniel Chávez González,* José Luis Aguirre Noyola, Néstor Daniel García Moreno, Gustavo Cuaxinque Flores, Yanet Romero Ramírez, Oscar Talavera Mendoza, Jeiry Toribio Jiménez. Universidad Autónoma de Guerrero |
| **BT-12** | **Hydrogen production by strain G088 psicrófilic using a dark fermentation process from agro-industrial waste.** *Sergio Cisneros de la Cueva,* Abraham M. Vidal Limón, Cecilia Lizeth Álvarez Guzmán, Víctor E. Balderas Hernández, Antonio De León Rodríguez. Instituto Potosino de Investigación Científica y Tecnológica, A.C. |
| **BT-13** | **Effect of textile dyes on growth of *Pleurotus ostreatus* and production of dye peroxidase.** *Jorge Luis Cuamatzi Flores,* Soley Berenice Nava Galicia, Edgardo Ulises Esquivel Naranjo, Martha Dolores Bibbins Martínez. Centro de Investigación en Biotecnología Aplicada (CIBA IPN Tlaxcala) |
| **BT-14** | **Chlorophyll A extraction from Arthrospira platensis and evaluation of its activity as photosensitizing agent in *Staphylococcus aureus*.** *Brenda Gisella Curiel Olaue*, Margarita Cid Hernández, Raúl Ricardo Quiñones López, Fermín Paúl Pacheco Moisés, Gilberto Velázquez Juárez, Sandra Fabiola Velasco Ramírez, Fernando Antonio López Dellamary Toral, Ricardo Manríquez González, Ana Cristina Ramírez Anguiano. Universidad de Guadalajara |

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| **BT-15** | **Functional analysis of a red-far red light photoreceptor in the filamentous fungus *Trichoderma atroviride*.** *Vincent Anthony Czarnowski Corona,* Alfredo Herrera Estrella, Edgardo Ulises Esquivel Naranjo. Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro |
| **BT-16** | **Antagonistic potential of bacteria isolated from agricultural soils in the state of Nayarit against pathogenic fungi of agronomic importance.** *Zulma Carolina Díaz Armenta,* Octavio Jonathan Cambero Campos, Carlos Rubén Carbajal Cazola, Adela Yolanda Bueno Durán, Jackeline Lizzeta Arvizu Gómez. Universidad Autónoma de Nayarit |
| **BT-17** | **Crecimiento de *Pleurotusostreatus* ATCC 32783 y actividad de lacasa intracelular desarrollado a diferente pH inicial de desarrollo en fermentación líquida.** *Rubén Díaz Godínez,* Carmen Sánchez, Gerardo Díaz Godínez. Centro de Investigación en Ciencias Biológicas, Universidad Autónoma de Tlaxcala |
| **BT-18** | **Streptomycin detection in wastewater by dot blot.** *Julieta Domínguez Soberanes,* Eva M. Salinas Miralles, Juan Jáuregui Rincón, Ivonne Chaides Zuñiga, Ileana E. Medina Ramírez, Norma A. Chávez Vela. Centro de Ciencias Básicas, Universidad Autónoma de Aguascalientes |
| **BT-19** | **Biopreservation of food pork ready to eat meat by lactic acid bacteria.** *Julieta Domínguez Soberanes,* Norma A. Chávez Vela, Héctor B. Escalona Buendía, Gabriela M. Rodríguez Serrano. Universidad Panamericana. Campus Aguascalientes |
| **BT-20** | **Identification of structural genes of enterocin 29α and enterocin 29β in the operon of the enterocin A produced by the strain *Enterococcus faecium* MXVK29.** *Edson Escamilla Martínez***,** Álvarez Cisneros YM, Fernández FJ, Ponce Alquicira E. Universidad Autónoma Metropolitana, Unidad Iztapalapa |
| **BT-21** | **Physiological effects caused by reduction on glucose uptake capacity in *Escherichia coli* mutant strains devoid of glucose transport related genes.** *Juan Carlos Fragoso Jiménez***,** Luz María Martínez Mejía, Noemi Flores Mejía, Georgina Hernández Chávez, Alfredo Martínez Jiménez, Guillermo Gosset Lagarda, Instituto de Biotecnología, UNAM |
| **BT-22** | **Growth assessment of *Rhizobium sp*. in minimal medium with glucose or succinate as carbon sources.** *Ramsés Ismael García Cabrera,* Norma A. Valdez Cruz, Mauricio A. Trujillo Roldán. Instituto de Investigaciones Biomédicas, UNAM |
| **BT-23** | **Cytotoxic activity on leukemia cell lines of exopolysaccharides and intra polysaccharides from *Humphreya coffeata* (Berk) Stey.** *Monserrat García García,* Norma A. Valdez Cruz, Mauricio A. Trujillo Roldán. Instituto de Investigaciones Biomédicas, UNAM |
| **BT-24** | **Oxygen concentration in culture of *Metarhizium anisopliae* generates conidia with sustained antioxidant activity.** *García Ortíz Nohemí,* Ernesto Favela Torres, Octavio Loera Corral. Universidad Autónoma Metropolitana, Unidad Iztapalapa |
| **BT-25** | **Molecular chaperone ZmFES1a: cisgenic over-expression to improve thermo tolerance.** *Verónica Garrocho Villegas,* Sabina Velázquez Márquez, Luis C Castillo, Estela Sánchez de Jiménez. Facultad de Química, UNAM |
| **BT-26** | **Autoreplicative plasmids for genetic engineering in *Lachancea kluyveri*.** *Nicolás Gómez Hernández,* Javier Israel Montalvo Arredondo, Lina Raquel Riego Ruiz. Instituto Potosino de Investigación Científica y Tecnológica, A.C. |
| **BT-27** | **Structural characterization of bacteriophage M13 in acid pH values.** *Jhoana L González Cansino,* Francisco Reyes Espinosa, L Irais Vera Robles, Andrés Hernández Arana. Universidad Autónoma Metropolitana, Unidad Iztapalapa |
| **BT-28** | **Laccase activity and specific growth rate of *Pleurotus ostreatus* NRRL 3526 grown on solid-sate fermentation and submerged fermentation.** *Maribel González Palma,* Carmen Sánchez, Gerardo Díaz Godínez, Daniel Martínez Carrera, Rubén Díaz Godínez. Centro de Investigación en Ciencias Biológicas, Universidad Autónoma de Tlaxcala |
| **BT-29** | **Promoter expression pchiAendochitinase chiA74 compared with two dependent promoters sporulation in *Bacillus thuringiensis.*** *Karen Stephania González Ponce***,** Luz Edith Casados Vázquez, José Eleazar Barboza Corona. Universidad de Guanajuato |

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| **BT-30** | **The importance of pH variation in different growth culture media during the production of recombinant proteins in *Escherichia coli*.** *Jesús Guerra Muñiz,* Mauricio A. Trujillo Roldán, Norma A. Valdez Cruz. Instituto de Investigaciones Biomédicas, UNAM |
| **BT-31** | **Monitoring of volatile organic compounds (VOCs) during plant-microorganism interaction in real time.** *Héctor Guillén Alonso,* Robert Winkler. CINVESTAV Unidad Irapuato |
| **BT-32** | **Cloning and expression of recombinant J1-1 defensin from *Capsicum chinense* in *Escherichia coli*.** *Francisco Guillén Chable,* Adolfo Guzmán, Georgina Estrada. Centro de Investigación Científica de Yucatán A.C. |
| **BT-33** | **Metabolic engineering for increasing the production of rhamnolipids in a *Pseudomonas aeruginosa* strain.** *Uriel Gutiérrez Gómez,* Gloria Soberón Chávez. Instituto de Investigaciones Biomédicas, UNAM |
| **BT-34** | **Biological effects of cyclodipeptides from *Pseudomonas aeruginosa* PAO1 in human cancer cells** *Laura Hernández Padilla,* Lorena Farías Rosales, Luis Alberto Sánchez Briones, Víctor Meza Carmen, Jesús Campos García. Universidad Michoacana de San Nicolás de Hidalgo |
| **BT-35** | **Caracterization of composts made from organic waste of Tepetitla de Lardizabal, Tlaxcala.** *Joseph Israel Hernández Rivadeneyra,* María Myrna Solís Oba, Rigoberto Castro Rivera. Centro de Investigación en Biotecnología Aplicada, IPN |
| **BT-36** | **Immunogenic properties of ectodomain of porcine rubulavirus Hemagglutinin-Neuraminidase produced in the yeast *Pichia pastoris*.** *Irma Herrera Camacho,* José Luis Cerriteño Sánchez, Gerardo Santos López, Julio Reyes Leyva, Lourdes Millán Pérez Peña, Nora Hilda Rosas Murrieta. ICUAP, BUAP |
| **BT-37** | **Critical path for obtaining nutraceuticals species of Rubus spp., and their relationship in preventing metabolic diseases.** *José Trinidad Herrera Pérez,* Rafael Ortiz Alvarado, Victor Meza Carmen, J. Carlos Santiago Jiménez. Universidad Michoacana de San Nicolás de Hidalgo |
| **BT-38** | **Recombinant overexpression of trypsin III mutants from Monterey sardine (*Sardinops sagax caerulea*).** *Nallely Hoyos González,* Manuel Ignacio Carretas Valdez, Aldo A. Arvizu Flores. Universidad de Sonora |
| **BT-39** | **Induction of *in vitro* morphogenetic response of *Kalanchoe daigremontiana* for the production of secondary metabolites.** *Anais Lizbeth Ibarra Chávez,*Taurino Méndez Díaz, Manuel Velázquez Ponce, Héctor G. Núñez Palenius, Lisset Herrera Isidrón. Instituto Politécnico Nacional |
| **BT-40** | ***In vitro* micropropagation of *Echino cactus grusonii* (Golden Barrel Cactus).** *Anais Lizbeth Ibarra Chávez,* Porfirio A. Gallegos Casillas, Juan C. Rodríguez Sierra, Luis E. Murillo Yáñez, Héctor G. Núñez Palenius, Lisset Herrera Isidrón. Instituto Politécnico Nacional |
| **BT-41** | **Determination of laccase and peroxidase activities of the moderate halophile *Aspergillus caesiellus* growing on phenanthrene and benzo(a)pyrene as only source of carbon.** *Martín Romualdo Ide Pérez,* Jorge Luis Folch Mallol, Ramón Batista García, María del Rayo Sánchez Carbente. Universidad Autónoma del Estado de Morelos |
| **BT-42** | **Evaluation of benzimidazoles as an alternative in staining of nucleic acids in agarose gel electrophoresis.** *María Teresa Izaguirre Hernández,* Manuel Velázquez Ponce, Karla Lizbeth Macías Sánchez, César Aza González. Unid. Profesional Interdisciplinaria de Ingeniería Campus Guanajuato, IPN |
| **BT-43** | **Protein engineering for molecular recognition; the cysteine protease inhibitor 1 of *Entamoeba histolytica*.** *Pedro Jiménez Sandoval,* Corina Díaz Quezada, Ezequiel Alejandro Madrigal Carrillo, Hugo Aníbal Santamaría Suárez, Alfredo Torres Larios, Luis G. Brieba. LANGEBIO, CINVESTAV, Unidad Irapuato |
| **BT-44** | **Study of the effect of ammonium on the expression of genes coding for regulators sRNAs of Rsm Family in *Azotobacter vinelandii*.** *Victoria Juárez Fuentes,* Liliana López Pliego, Miguel Castañeda Lucio. Instituto de Microbiología, Benemérita Universidad Autónoma de Puebla |
| **BT-45** | **Analysis of the structural domains of ChiA74 chitin *Bacillus thuringiensis*.** *Estefanía O. Juárez Hernández,* Luz Edith Casados Vázquez, José Eleazar Barboza Corona. Universidad de Guanajuato |
| **BT-46** | **The methylotrophic yeast *Pichia pastoris* is a suitable host to express complex antigens from *Mycobacterium tuberculosis*.** *Daniel Juárez López,* Clarita Olvera, Carlos Giroshi Bando Campos, Francisco Javier García Monroy, Mauricio Alberto Trujillo Roldán, Norma Adriana Valdez Cruz. Instituto de Investigaciones Biomédicas, UNAM |

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| **BT-47** | **Expression of *Vitreoscilla stercoriaria* hemoglobin improves growth and reduces lactate yields in CHO-K1 cells.** *Mariana Juárez Osorio,* Claudia Haydée González de la Rosa, Álvaro R. Lara. Universidad Autónoma Metropolitana, Unidad Cuajimalpa |
| **BT-48** | **Analysis of the role of the peroxidase ZmPrx35: an insight to the biochemical mechanisms of post-harvest insect resistance in maize (*Zea mays L*).** *Laura Margarita López Castillo,* María Fernanda Díaz Flores Rivera, Silverio García Lara. Instituto Tecnológico de Estudios Superiores de Monterrey |
| **BT-49** | **Immobilized laccase-mediated free radical polymerization of acrylamide in deep eutectic solvents.** *Héctor Alexander López Muñoz,* Claudia Ivonne Muñoz Sánchez, Ma. Irma Cristina Pérez Pérez, Sandra Herrera Pérez, Gabriel Luna Bárcenas, Francisco Villaseñor Ortega. Instituto Tecnológico de Celaya |
| **BT-50** | **Stabilizing effect of the V915L mutation on the reductase domain of engineered cytochrome P450 BM-3.** *Jair López Sánchez,* Valeria Guzmán Luna, Gloria Saab Rincón. Instituto de Biotecnología, UNAM |
| **BT-51** | **Enzymatic profiling of oxidases produced by *Oxyporus latermaginatus* in submerged fermentation in the presence of lignocellulosic.** *Edy Manuel Surian Cruz,* Soley Berenice Nava Galicia, Martha Bibbins Martínez. Centro de Investigación en Biotecnología Aplicada, IPN |
| **BT-52** | **Study of scale-up criterion kLa and Pg/V in bioprocess for recombinant expression of dextranase in *Pichia pastoris*.** *Miguel Ángel Marín Muñoz,* Juan Jáuregui Rincón, Leobardo Serrano Carreón, Norma Angélica Chávez Vela. Universidad Autónoma de Aguascalientes |
| **BT-53** | **Nanovesicles from eukaryotic cells produced by acoustic cavitation.** *Gisela Martínez Andrade,* Blanca E. Millán Chiu, Susana Vargas, Francisco Fernández, Achim M. Loske, Luz M. López Marín. Centro de Física Aplicada y Tecnología Avanzada, UNAM |
| **BT-54** | **Production and purification of the proteins NEP y NS1 of influenza AH1N1 virus in *E. coli*.** *Marbeht Martínez Castañeda,* José Luis Cerriteño Sánchez, Nora Rosas Murrieta, Julio Reyes Leyva, Irma Herrera Camacho. Centro de Química-ICUAP, Universidad Autónoma de Puebla |
| **BT-55** | **Cloning and Expression of mutant defensins with specific binding to phosphatidic acid.***Cynthia Gabriela Martínez Liu,* Adolfo Guzmán Antonio, Georgina Estrada. Centro de Investigación Científica de Yucatán A.C. |
| **BT-56** | **Study and bioinformatic characterization of *omp6*, *omp7*, *omp8*, *omp9*, *omp10* genes from Mexican strains of *Anaplasma marginale*.** *Julián Martínez Salgado,* Itzel Amaro Estrada, Rosa Estela Quiroz Castañeda, Sergio D. Rodríguez Camarillo. Centro Nacional de Investigación Disciplinaria en Parasitología Veterinaria, INIFAP |
| **BT-57** | **Isolation of hydrocarbon degrading bacteria from diesel and gasoline contaminated soil at La Comarca Lagunera.** *José Antonio Martínez Villalba,* Iris Estefanía Moreno García, Mariana Guadalupe Jiménez Valdez, Inty Omar Hernández De Lira, Salvador Sánchez Muñoz. Universidad Iberoamericana Torreón |
| **BT-58** | ***Solanum lycopersicum* interaction with a *Trichoderma atroviride* strain that overexpresses an expansin type protein.** *Olivia Martínez Villamil,* Richa Mehta, Edgar Balcázar López, Karina Atriztán Hernández, María del Rayo Sánchez Carbente, Ayixón Sánchez Reyes, Carlos Alberto González Chávez, Verónica Lira Ruan, Alfredo Herrera Estrella, Jorge Luis Folch Mallol. UAEM |
| **BT-59** | **Study of the interaction and permeability originated by PLGA nanoparticles loaded with melittin in phospholipid membranes that resembles mammary epithelial and breast cancer cells.***Arely Matamoros Acosta,* Abelardo Chávez Montes, Dvorak Montiel Condado, Brenda González Hernández, Azucena González Horta. Universidad Autónoma de Nuevo León |
| **BT-60** | **Study of the overexpression effect of the RsmsRNAs and its effects in alginate production in *Azotobacter vinelandii*.** *Gabriela Fernanda May Compañ,* Liliana López Pliego, Miguel Castañeda Lucio. Instituto de Microbiología, Universidad Autónoma de Puebla |
| **BT-61** | **Characterization of native chitinases from marine microorganisms and their possible application in biotechnology in biological control.** *Mazón Román Luis Enrique,* Licea Navarro Alexei, Martínez Morales Fernando, Trejo Hernández María del Refugio. Universidad Autónoma del Estado de Morelos |

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| **BT-62** | **Prolactin and 17β-estradiol induce pro-Inflammatory cytokines in bovine mammary epithelial cells inhibiting *Staphylococcus aureus* Internalization.** *Ricardo Iván Medina Estrada,* Joel Edmundo López Meza, Alejandra Ochoa Zarzosa. Universidad Michoacana de San Nicolás de Hidalgo |
| **BT-63** | **Elicitors for the production of esteviosides from cells suspension of *Stevia rebaudiana* Bertoni.** *Lizbeth Mejía Espejel,* Alejandrina Robledo Paz, Edmundo Lozoya Gloria. Colegio de Postgraduados |
| **BT-64** | **PepGMV tolerance induction in pepper plants with hydrogen peroxide solutions.** *Laura Mejía Teniente,* González Chavira Mario Martin, Acosta García Gerardo, Torres Pacheco Irineo, Guevara González Ramón Gerardo. Universidad de Guanajuato |
| **BT-65** | **Evaluation of the modulatory effect of peptides with affinity to human protein disulfide isomerase (PDIA1).** *Alexis Zarahy Minchaca Acosta,* Ramón Castellanos Martínez, Rosa Elena Mares Alejandre, Marco Antonio Ramos Ibarra. Facultad de Ciencias Químicas e Ingenieria, Universidad Autónoma de Baja California |
| **BT-66** | **Metabolic signatures of tomato plants with differences in Jasmonate Biosynthesis.** *Josaphat Miguel Montero Vargas,* Kena Casarrubias Castillo, John Délano Frier, Robert Winkler. CINVESTAV Unidad Irapuato |
| **BT-67** | **Continuous monitoring of volatile organic compounds (VOCs) emitted by tomato plants (*Solanum lycopersicum*) during their interaction with pathogens.** *Abigail Moreno Pedraza,* Robert Winkler. CINVESTAV Unidad Irapuato |
| **BT-68** | **Determination of lignocellulolytic enzyme activity of the moderate halophile fungus *Aspergillus caesiellus* growing in wheat straw and agave fibers in halophilic conditions.** *Víctor Manuel Ocampo Medina,* Ramón Batista García, Eya Caridad Rodríguez Pupo, Ayixón Sánchez Reyes, Andrés Zárate Romero, María del Rocío Rodríguez Hernández, Jorge Luis Folch Mallol, María del Rayo Sánchez Carbente. Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos |
| **BT-69** | **Visualizing calcium nano domains in living cells through optical patch- clamp recording.** *José Pablo Ocelotl Oviedo,* Adán O. Guerrero Cárdenas, Alberto Darszon. Instituto de Biotecnología, UNAM |
| **BT-70** | **Roles of enzyme groups within each region of the intestinal tract of *E. fetida* during vermi composting.** *Berenice Ordoñez Arévalo,* Karina Guillén Navarro, Esperanza Huerta, Raúl Cuevas, María de los Ángeles Calixto Romo. El Colegio de la Frontera Sur, Unidad Tapachula |
| **BT-71** | **Comparison of three different pH on recombinant *E. coli* BL21 (DE3) cultivation.** *Manuel Ortega Hernando,* Alejandro Olvera, Alejandro Alagón, Mauricio A. Trujillo Roldán, Norma A. Valdez Cruz. Instituto de Investigaciones Biomédicas, UNAM |
| **BT-72** | **Crude extracts of broccoli (*Brassica oleracea var. Italica*) have inhibitory effect against bacteria of importance in human health and fungi.** *Rubén Darío Pacheco Cano,* Blanca Estela García Almendárez, Rubén Salcedo Hernández, Gustavo Hernández Guzmán, José Eleazar Barboza Corona. Universidad de Guanajuato |
| **BT-73** | **Biomolecular interaction analysis using magnetic particles.** *Ana María Peña Balderas,* Rosario Esperanza Moctezuma Martiñon, Vanesa Olivares Illana. Instituto de Física, Universidad Autónoma de San Luis Potosí |
| **BT-74** | **Functional analysis of a 30 kDa endochitinase by VIGS of *S. habrochaites* and *S. arcanum* species, to evaluate its possible role in defense against the bacterial canker.** *Leonardo Isaac Pereyra Bistraín,* Mayra Janeth Esparza Araiza, José Pablo Lara Ávila, Rosalba Castillo Collazo, Dulce Alejandra Rougon Cardoso, Ángel Gabriel Alpuche Solís. IPICyT |
| **BT-75** | **Spores production by Bacillis thuringenis grown under solid-state and submerged culture.** *Daniel Guadalupe Pérez Solís,* Ernesto Favela Torres, Gustavo Viniegra González. Universidad de Ciencias y Artes de Chiapas |
| **BT-76** | **Functional expression of a Bowman-Birk inhibitor from Tepary bean seeds and atypical interaction analysis with bovine chymotrypsin.** *Raquel Pliego Arreaga,* Octavio Roldán Padrón, Elizabeth Mendiola Olaya, Luis Brieba, Alejandro Blanco Labra. CINVESTAV Unidad Irapuato |
| **BT-77** | **Design of a chimeric gene with antimicrobial activity derived from *Moringa oleifera* Lam. and its expression in *Chlamydomonas reinhardtiii*.** *Quezada Rivera Jesús Josafath,* Soria Guerra Ruth Elena, Morales Domínguez José Francisco. Universidad Autónoma de Aguascalientes |

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| **BT-78** | **Chemical crosslinking of nitrilase filaments for develop a stable and reusable biocatalyst.** *Julio César Quintana Rojas,* Georgina Garza Ramos Martínez. Departamento de Bioquímica, Facultad de Medicina, UNAM |
| **BT-79** | **Effect of vermicompost on the lettuce innocuity.** *Víctor Hugo Ramos García,* Rebeca Flores Magallón, Gilberto Vázquez Gálvez, Juan Manuel González Prieto. Centro de Biotecnología Genómica, IPN |
| **BT-80** | **Cytotoxic activity of *Thevetiaperuviana* extract against cancer cell lines.** *José Alberto Ramos Silva,* Faviola Isabel Tavares Carreón, Yesenia Cristal García Silva, Susana De la Torre Zavala, Aída Rodríguez García, Hamlet Avilés Arnaut. Facultad de Ciencias Biológicas, UANL |
| **BT-81** | **Evaluation of molecular and metabolic events during recombinant protein production in *Escherichia coli* with a thermo-inducible system.** *Sara Restrepo Pineda,* Carlos Giroshi Bando Campos, Norma Adriana Valdez Cruz, Mauricio Alberto Trujillo Roldán. Instituto de Investigaciones Biomédicas, UNAM |
| **BT-82** | **Overexpression of human cystatin C in *E. coli* and its purification; Isolation of recombinant protein that has been contaminated by nucleic acid after breaking the cells.** *Francisco Reyes Espinosa,* L. Irais Vera Robles, Andrés Hernández Arana. Universidad Autónoma Metropolitana. Unidad Iztapalapa |
| **BT 83** | **Study of the histidine kinase RetS over the expression of the small regulatory RNAs of the family *rsm* in *Azotobacter vinelandii*.** *Jimena Itzel Reyes Nicolau,* Liliana López Pliego, Miguel Castañeda Lucio. Instituto de Microbiología, Universidad Autónoma de Puebla |
| **BT-84** | **Determination of antimicrobial activity of *SPC13*, antimicrobial peptide isolated from the venom of *Scolopendra polymorpha*, against gram-negative bacteria.** *Carmen Itzamatul Rodríguez Alejandro,* Ma. Del Carmen Gutiérrez Villafuerte, Lucero Valladares Cisneros. Universidad Autónoma del Estado de Morelos |
| **BT-85** | **Characterization of lead compounds bioaccumulated by bacterial strains isolated from metal contaminated soil.** *Viridiana Rodríguez Sánchez,* Vicente Rodríguez González, María Leticia Pérez Arrieta, Juan Armando Flores de la Torre, Jesús Guzmán Moreno, Rosa María Ramírez Santoyo, Luz Elena Vidales Rodríguez. Universidad Autónoma de Zacatecas |
| **BT-86** | **Isolation and identification of metal tolerant filamentous fungi and their use in the synthesis of gold and silver nanoparticles.** *Candelario Rodríguez Serrano,* Jesús Guzmán Moreno, Rosa María Ramírez Santoyo, Vicente Rodríguez González, José Juan Ortega Sigala, Luz Elena Vidales Rodríguez. Universidad Autónoma de Zacatecas |
| **BT-87** | **Isolation of bacteria from extreme environments from Baja California Sur with antibacterial activity against multidrug-resistant *Staphylococcus aureus*.** *Gregorio Rodríguez Valdez,* Carlos Angulo. Centro de Investigaciones Biológicas del Noroeste S.C. |
| **BT-88** | **Isolation and characterization of levanase of *Clavibacter michiganensis subsp michiganensis*.** *Mario Raziel Romay Ramírez,* Jesús Hernández Romano, Luis Gerardo Treviño Quintanilla, Sandra Morales Arrieta. Universidad Politécnica del Estado de Morelos |
| **BT-89** | **Implementation of a new molecular tool to generate multiple mutants in *Azotobacter vinelandii*.** *Araceli Rosales Cruz,* Liliana López Pliego, Miguel Castañeda Lucio. Instituto de Microbiología, Universidad Autónoma de Puebla |
| **BT-90** | **Identification and characterization of heavy metal tolerant endophyte fungi from *Acacia farneciana*.** *Salazar Ramírez Giovanni*, Folch Mallol Jorge L, Mussali Galante Patricia. UAEM |
| **BT-91** | **Evaluation of bacterial endophyte strains of legumes as plant growth promoting rhizobacteria.** *Ricardo Sánchez Cruz,* Jorge Luis Folch Mallol, Arnoldo Wong Villarreal, Ayixon Sánchez Reyes. Universidad Autónoma del Estado de Morelos |
| **BT-92** | **Characterization of microorganisms with biotechnological potential isolated from sites contaminated with heavy metals.** *Juan Francisco Sánchez López,* Miryam Jhazmin Torres Gómez, Ana Laura Rodríguez Sotelo, Agustín Hilario Rocha Ramírez, Anastasio Cortés Mendoza, Juan de Dios Ortiz Alvarado, Aurelio Álvarez Vargas, Carmen Cano Canchola. Unidad Profesional Interdisciplinaria de Ingeniería, Campus Guanajuato, IPN |

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| **BT-93** | **Partial purification and characterization of milk clotting enzyme from *Moringa oleifera* Lam.** *María A. Sánchez Muñoz,* Mónica A. Valdez Solana, Claudia Avitia Domínguez C, Alfredo Téllez Valencia, Juan R Esparza Rivera, Jorge A Meza Velázquez, Patricia Ramírez Baca, Erick Sierra Campos. Universidad Juárez del Estado de Durango |
| **BT-94** | **Study of the genes involved in the biosynthesis of Cyclodipeptides; cyclo(L-Pro-L-Tyr), cyclo(L-Pro-L-Phe) and cyclo(L-Pro-L-Val) from *Pseudomonas aeruginosa* PAO1.** *Cristhian Said Solís Ortíz,* Rodrigo García López, Laura Hernández Padilla, Alma Díaz Pérez, Jesús Campos García. Universidad Michoacana de San Nicolás de Hidalgo |
| **BT-95** | **Effect of hyperoxidising atmosphere on UVB radiation resistance and its relationship with the activity of the enzyme Gpx in conidia of two strains *I. fumosorosea*.** *Gerardo Suárez Vergel,* Nohemí García Ortiz, Paul Misael Garza López, José Francisco Miranda Hernández, Octavio Loera Corral. Universidad Autónoma Metropolitana Unidad Iztapalapa |
| **BT-96** | **Expression of amebic chitinase (*Eh*CHT1) on the surface of *Escherichia coli*.** *Ricardo Torres Bañaga,* Patricia Lilián Alejandra Muñoz Muñoz, Rosa Elena Mares Alejandre, José Luis Mijangos Montiel, Samuel Guillermo Meléndez López, Marco Antonio Ramos Ibarra. Facultad de Ciencias Químicas e Ingeniería, Universidad Autónoma de Baja California |
| **BT-97** | **Binding Calorimetric Study of a Stabilized Human Cystatin C and Chymopapain.** *David Octavio Tovar Anaya,* María Teresa Vieyra Eusebio, Liliana Irais Vera Robles, Ponciano García Gutiérrez y Rafael A. Zubillaga. Universidad Autónoma Metropolitana Unidad Iztapalapa |
| **BT-98** | ***In vitro* propagation of pineapple (*Ananas comosus* (L.) Merr.) cv. 'Smooth Cayenne'.** *Erik Noé Tovar Peralta,* Martin Peralta Gil, José Silvestre Delgadillo Díaz de León, Eugenio Pérez Molphe Balch. Universidad Autónoma de Aguascalientes |
| **BT-99** | **Inulinases as an alternative to the use of agave and its wastes to produce fructose and fructo oligosaccharides.** *Jonathan Trapala Reyna,* Carmina Montiel Pacheco, Eduardo Bárzana García, Sandra Pérez Munguía. Facultad de Química, UNAM |
| **BT-100** | **Genomic sequencing and characterization of the metabolic pathway involved in chloranilic acid degradation on *Herbaspirillum* sp. TQ07.** *Luis Gerardo Treviño Quintanilla,* Itzel López Mendoza, Juan José Colín Salinas. Universidad Politécnica del Estado de Morelos |
| **BT-101** | **Degradation of pyrene and phenanthrene in hypersaline conditions by *Aspergillus caesiellus* H1 halophyla strain.** *Deborah González Abradelo,* María del Rayo Sánchez Carbente, Jorge Luis Folch Mallol, Verónica Lira Ruan, Ramón Alberto Batista García. Centro de Investigación en Dinámica Celular, Universidad Autónoma del Estado de Morelos |
| **BT-102** | **Identification and isolation of proteinaceous compounds with antimicrobial activity in the *Scolopendraviridis* Venom.** *Lucero Valladares Cisneros,* María del Carmen Gutiérrez Villafuerte. Universidad Autónoma del Estado de Morelos |
| **BT-103** | **Lytic activity of melittin on *Neochlorisoleo abundans* (Chlorophyta) for enhance neutral lipid extraction.** *Magda Patricia Vargas Pérez,* Azucena González Horta, Hugo Alberto Luna Olvera, Abelardo Chávez Montes. Facultad de Ciencias Químicas, Universidad Autónoma de Nuevo León |
| **BT-104** | **Cytotoxic effects and apoptosis induction by defensine gamma thionin (*Capsicum chinense*) and butyrate in colon cancer cell lines.** *María Elena Velázquez Hernández,* Alejandra Ochoa Zarzosa, Joel Edmundo López Meza. Universidad Michoacana de San Nicolás de Hidalgo |
| **BT-105** | **Maize differential tolerance on water stress conditions: Contribution of soluble solutes and dehydrins.** *Sabina Velázquez Márquez,* Verónica Garrocho Villegas, Víctor Gonzáles Hernández, Estela Sánchez Quintanar. Facultad de Química, UNAM |
| **BT-106** | **Cell surface display of proteins on filamentous fungi.** *Jorge Verdín,* Elisa Fernández Castillo, Laura Castillo Ortega, Samuel Atic Vargas, Jesús Urbar Ulloa. Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C. |
| **BT-107** | **A cytoplasmic Slo3 fragment regulates both Slo3 and Slo1 potassium channels in mouse.** *Alberto Vicens Sánchez,* David C. Wrighton, Karla Andrade, Julio César Chávez, Diego Cortez, Rosa María Gutiérrez, Jonathan D. Lippiat, Claudia Treviño. Instituto de Biotecnología, UNAM |

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| **BT-108** | **Molecular characterization of hydrogenase activity from an Antarctic biohydrogen producing strain.** *Abraham M. Vidal Limón,* Sergio Cisneros de la Cueva, Cecilia Lizeth Álvarez Guzmán, Víctor E. Balderas Hernández, Antonio De León Rodríguez. Instituto Potosino de Investigación Científica y Tecnológica, A.C. |
| **BT-109** | **Purification of a a-L-arabinofuranosidase from *Colletotrichum lindemuthianum* race 1472.** *María Guadalupe Villa Rivera,* María Guadalupe Zavala Páramo, Alicia Lara Márquez, Everardo López Romero, Ulises Conejo Saucedo, Horacio Cano Camacho. Universidad Michoacana de San Nicolás de Hidalgo |
| **BT-110** | **Implementation of a PCR-based system for generation of mutants in the phytopathogenic fungus *Ustilago maydis*.** *Nubia Andrea Villota Salazar,* Artemio Mendoza Mendoza, Juan Manuel González Prieto. Centro de Biotecnología Genómica, IPN |
| **BT-111** | **Speeding up the enzymatic reaction of triosephosphate isomerase.** *Vique Sánchez JL*, Caro Gómez LA, Brieba Luis, Romero Romero S, Fernández Velasco DA, Benítez Cardoza C. Escuela Nacional de Medicina y Homeopatía, IPN |
| **BT-112** | **Comparative analysis of the transcriptome of *Aspergillus caesiellus* grown in pyrene and phenanthrene in hypersaline conditions.** *Heidy Peidro Guzmán,* Yordanis Pérez Llano, María del Rayo Sánchez Carbente, Jorge Luis Folch Mallol, Ramón Alberto Batista García. Centro de Investigaciones en Dinámica Molecular |
| **BT-113** | **Preliminary study of thermoplastic starch biofilms with additives to inhibit the growth of *Salmonella and Escherichia coli* in blackberry.** *Cinthya Ofelda De la Rosa Morales*, José Luis Rivera Corona, Manuel Carrillo Morales and Sandra Morales Arrieta, Universidad Politécnica del Estado de Morelos |
| **BT-114** | **Synthesis of catechol melanin from glycerol employing engineered *Escherichia coli.*** *Ramón de Anda,* Alejandra Mejía Caballero, Georgina Hernández Chávez, Carlos Vargas, Simone Rogg, Alfredo Martinez, Francisco Bolívar, Victor M. Castaño and Guillermo Gosset. Departamento de Ingeniería Celular y Biocatálisis, Instituto de Biotecnología, UNAM |
| **BT-115** | **Plant volatiles act in the direct resistance to pathogenic fungi.** Luis E. Rivera Macías, Elizabeth Quintana Rodriguez, Rosa M. Adame Álvarez, Jorge Molina Torres and Martin Heil. *CINVESTAV* Unidad Irapuato. |
| **BT-116** | **Effect of polyphenols from the leaves of Mexican avocado (*Persea americana* var. drymifolia) over the gene expression of Methicillin-Resistant *Staphylococcus aureus*.** *Victor Eustorgio Aguirre Arzola*, Miguel Angel García Moreno, Guillermo Cristian Martinez Ávila, Ma del Carmen Ojeda Zacarías. Facultad de Agronomía, Universidad Autónoma de Nuevo Léon |

## MEDICINE, HEALTH AND NUTRITION

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| **M-1** | **Effect of mitochondrial inhibitors on the viability of cancer stem-like cells of breast cancer MCF-7.** *Alhelí Adán Ladrón de Guevara,* Juan Carlos Gallardo Pérez. Instituto Nacional de Cardiología |
| **M-2** | **Meniscal cell membrane express ectopic IF1-ATP synthase and its expression is regulated by TNF-α and IL-1β**. *Rocío Aguilar Gaytan*, Francisco Pérez Jiménez, Raúl Pichardo Bahena, Cecilia Zazueta Mendizábal, Clemente Ibarra. Instituto Nacional de Rehabilitación |
| **M-3** | **Establishment of an obesity model to evaluate hepatic steatosis**. *Clara Alba Betancourt*, Claudia Leticia Mendoza Macías, Martha Alicia Deveze Álvarez, Marco Antonio Ramírez Morales, Ángel Josabad Alonso Castro, Martha Citlalli Contreras Romo. Departamento de Farmacia. Div. De Ciencias Naturales y Exactas. Universidad de Guanajuato |
| **M-4** | **2-methoxyestradiol and dichloroacetate effects on A-549 and MRC5 cells grown under hypoxic conditions**. *Arnoldo Aquino Gálvez*, Jesús Eduardo Sánchez Calleja, Javier Delgado Tello, Criselda Mendoza Milla, Manuel Castillejos López, Luz María Torres Espíndola, Bettina Sommer Cervantes, Marco Checa Caratachea, Carlos Cabello Gutiérrez, Georgina González Ávila. Instituto Nacional de Enfermedades Respiratorias Ismael Cosio Villegas |

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| **M-5** | **Analysis of the expression of miR-15a, miR-16-1 and miR-193a in circulation and its correlation with the expression of WT1 in patients with nephrotic syndrome**. *Mariela Arellano Rodríguez***,** Pablo Zapata Benavides, Juan José Bollain y Goytia de la Rosa, Felipe de JesusTorres del Muro, Esperanza Avalos Díaz, Rafael Herrera Esparza, Cristina Rodríguez Padilla. Universidad Autónoma de Nuevo León |
| **M-6** | **Purification of human Paraoxonsae 1 (PON1h) from blood inhibits biofilm formation on *Aeromonascaviae* Sch3**. *Gabriel Betanzos Cabrera***,** Celaya Correa Iván Fco, Mercado Monroy José, Resendiz Otero María Fernanda, Ronces Arrieta Rodrigo, Ariza Ortega José Alberto, Estrada Luna Diego. Instituto de Ciencias de la Salud, Universidad Autónoma del Estado de Hidalgo |
| **M-7** | **Protein levels of IRE1, ATF6 and PERK (Unfolded Protein Response mediators) in alkali corneal lesion in rat.** *Rosalba Buenrostro León***,** Sindira González López, Raquel Guerrero Alba, José Rafael Villafan Bernal, Luis Torres Bernal, Eduardo Emmanuel Valdez Morales, Sugela Susana Blancas Zugarazo. Neurosurgery, Universidad Autónoma de Aguascalientes |
| **M-8** | **Effects of *Spirulina* maxima on physical performance and antioxidant status in athletes**. **Ma.** *Guadalupe Calderón Gallardo,* Marco Antonio Juárez Oropeza, María Teresa Espinosa García and Patricia Victoria Torres Durán. Bioquímica. Facultad de Medicina, UNAM |
| **M-9** | **Preliminary study of *Coriandrumsativum* seed consumption on dysglycemia and dyslipidemia models.** *Oscar De Jesús Calva Cruz***,** Samuel Treviño Mora, Violeta Aburto Luna, Roberto Sánchez Olea. Instituto de Física, Universidad Autónoma de San Luis Potosí |
| **M-10** | **Coxpression of progesterone receptor and TGF-β in epithelial Ovarian cancer**. *Calvillo Robledo Argelia***,** Gómora Herrera María José, Damian Matsumura Pablo, Morales Vásquez Flavia, Roman Bassaure E, Méndez Herrera Carmen, Pedernera Astegiano Enrique. Universidad Autónoma Metropolitana Unidad Iztapalapa |
| **M-11** | **Effect of *Moringa oleifera extract* on glutaminase-1 activity in a breast cancer murine model**. *Mara Ibeth Campos Almazán,* Jessica Lizbeth Hernández Rivera, Jaime Abraham de Lira Sánchez, Erick Sierra Campos, Mónica Andrea Valdéz Solana, Claudia Isela Avitia Domínguez, Alfredo Téllez Valencia. Facultad de Medicina y Nutrición, Universidad Juárez del Estado de Durango |
| **M-12** | **Antagonistic activity of a Lactobacillus sp strain isolated from environment against bacteria associated with foodborne diseases**. *Ricardo Carrasco Torres***,** Juan Carlos Benítez Serrano, Azarel Ruíz Román, Patricia Aguilar Alonso, Gloria León Tello, Laura Martínez Pérez. Depto. de Microbiología. Facultad de Cs. Químicas, BUAP |
| **M-13** | **Hypoxia modulates the expression of the cytochrome P450 isoforms CYP1A1, CYP2S1, CYP3A7 and CYP24A1 in cancer.** *Rosa Angélica Castillo Rodríguez***,** Sergio Juárez Méndez, Víctor Manuel Dávila-Borja. Laboratorio de Oncología Experimental, Instituto Nacional de Pediatría |
| **M-14** | **MicroRNAs profiling in breast cancer stem cells-like ($CD44^+/CD24^-$) identify members of WNT/b-catenin signaling pathway as targets of let-7c-3p**. *Karen Nayeli Chaparro Pulido*, Lizeth Fuentes Mera, Claudia Villarreal Ovalle, Vanessa Pérez Silos, Erika Coronado Cerda, Elsa Maribel Aguilar Medina, Rosalio Ramos Payán, Laurence Annie Marchat, César López Camarillo. Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa |
| **M-15** | **In the HCC827 lung adenocarcinoma cell line, Erlotinib induces apoptosis associated with the expression of immunogenic cell death markers**. *Rodolfo Luis Chávez Domínguez***,** Dolores Aguilar Chazares, Heriberto Prado García, Lorenzo Islas Vázquez, José Sullivan López González. Instituto Nacional de Enfermedades Respiratorias Ismael Cosio Villegas |
| **M-16** | **Regulation of respiratory chain by *Moringa oleifera* leaves extract in a breast cancer murine model.** *Jaime Abraham De Lira Sánchez***,** Mara Ibeth Campos Almazán, Jessica Lizbeth Hernández Rivera, Mónica Andrea Valdés Solana, Erick Sierra Campos, Alfredo Téllez Valencia, Claudia Isela Avitia Domínguez. Facultad de Medicina y Nutrición, Universidad Juárez del Estado de Durango |
| **M-17** | **(-)-Epicatechin flavonoid treatment improves the mechanical heart performance in healthy mice.** *Sergio De los Santos Enríquez***,** Carlos Jesús González Gutiérrez, Sauri Hernández Resendiz, Carlos Palma Flores, Jennifer Alcántara Blancarte, Cecilia Zazueta, Alejandro Zentella, Patricia Canto, Ramón Coral Vázquez. Instituto de Investigaciones Biomédicas, UNAM |

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| **M-18** | **Effect of supplementation of dehydrated pineapple core (*Ananascomosus*) in glycemic index and fiber content in a bisquet type bread**. *Karina Nathalie Escutia López***,** Lourdes Mendoza Ruíz, Natalia Cecilia Hernández Delgado, Luis Fernando Cervantes Serrato, Eugenia Samantha Hidalgo Gutiérrez, Esbeydy Enríquez Guerra, Epifanio Jiménez García, Ma. Elena Sánchez Pardo. Unidad Profesional Adolfo López Mateos. Instituto Politécnico Nacional |
| **M-19** | **Effects of damiana (*T. diffusa Willd. vardiffusa*) in diabetes lipids control.** *Esquivel Gutiérrez Edgar Romualdo***,** Alcaraz Meléndez Lilia, Hernández Herrera Roberto. Centro de Investigaciones Biológicas Del Noroeste S.C. |
| **M-20** | **Daily supplementation with fresh pomegranate juice increases paraoxonase 1 expression and activity in mice fed an atherogenic diet**. *Estrada Luna D,* Martínez Hinojosa E, Cancino Diaz JC, Belefant Miller H, López Rodríguez G., BetanzosCabrera G. Instituto de Ciencias de la Salud, Universidad Autónoma del Estado de Hidalgo |
| **M-21** | **Finding potential inhibitors of shikimate kinase from methicillin resistant *Staphylococcus aureus* through virtual screening**. *Alejandro Favela Candia***,** Rafael Alejandro Moreno Silerio, Jorge Cisneros Martínez, Marcelo Gémez Palacio Gatelum, Claudia Isela Avitia Domínguez, Alfredo Téllez Valencia. Universidad Juárez del Estado de Durango |
| **M-22** | **Characterization of a type 2 diabetes model in lactating Wistar rats**. *María del Consuelo Figueroa García***,** Ricardo Mejía Zepeda. FESI. UNAM |
| **M-23** | **Extraction and characterization of protein fractions with nutraceutical potential from two species of insects of Tenebrionidae family: *Ulomoides dermestoides and Tenebrio molitor*.** *Flores Mendoza Daniel Rogelio,* Vania Montserrat Salazar Camarillo, Ramírez Anguiano Ana Cristina, Gallegos Castillo Saúl, José de Jesús Luna Díaz, Navarrete Heredia José Luis, Velasco Ramírez Sandra Fabiola, Velázquez Juárez Gilberto. Departamento de Química. Centro Universitario de Ciencias Exactas e Ingenierías. Universidad de Guadalajara |
| **M-24** | **FAT1 in idiopathic pulmonary fibrosis.** *García Álvarez Juna Antonio***,** Ferrer A, Medrano L, Gutiérrez J, Ramírez R, Medina D, Balderas Y, Iglesias A, Selman M, Pardo A. Laboratorio de Bioquímica, Facultad de Ciencias, UNAM |
| **M-25** | **2D-DIGE analysis of serum of Mexican patients with Type-2 diabetes in relation to their body mass index**. *Erik Elvin Gómez Cardona***,** Eric Edmundo Hernández Domínguez, Alberto Barrera Pacheco, Aida Jimena Velarde Salcedo, Antonio de León Rodríguez, Agustín Díaz Gois, Ana Paulina Barba de la Rosa. Instituto Potosino de Investigación Científica y Tecnológica |
| **M-26** | **Antimicrobial and Antibiofilm effect of Flavonoids in Periodonto pathogens**. *Juan Arturo Gómez Mora***,** Marco Antonio Meraz Rodríguez, Mónica Arisbet Flores Sánchez, Gloria Gutiérrez Venegas. Laboratorio de Bioquímica. División de Estudios de Posgrado e Investigación. Facultad de Odontología, UNAM |
| **M-27** | **Expression of opioid grow factor receptor (OGFr) and transient receptor potential vanilloid 1 (TRPV1) in a rat alkali-burned cornea model**. *Sindira González López***,** Rosalba Buenrostro, Luis Fernando Torres Bernal, Rafael Villafan Bernal, Sugela Susana Blancas Zugarazo, Eduardo Emmanuel Valdez Morales. Universidad Autónoma de Aguascalientes |
| **M-28** | **Endothelial activation mediated by tumor soluble factors secreted by breast cancer cell lines with low and high metastatic potential.** *César Alejandro Guzmán Pérez***,** Alfredo Ibarra Sánchez, Alberto José Cabrera Quintero, Juan Pablo Aragón Hernández, Jorge Román Audifred Salomón, José Luis Ventura Gallegos, Claudia González Espinosa, Alejandro Zentella Dehesa. Unidad Bioquímica. Instituto Nacional de Ciencias Médicas y Nutrición |
| **M-29** | **PCR identification for the four most common *Candida* species in nosocomial infections**. *José Oscar Arturo Hernández Carreón***,** Grecia Hernández Hernández, Alejandro de las Peñas Nava, Irene Castaño Navarro. Instituto Potosino de Investigación Científica y Tecnológica |
| **M-30** | **Development of functional gummies using reduced moisture xoconostle (*Opuntia joconostle*) pulp** *Natalia Cecilia Hernández Delgado***,** Paola Meza Crispín, Karina Nathalie Escutia López, Luis Fernando Cervantes Serrato, Esbeydy Enríquez Guerra, Eugenia Samanta Hidalgo Gutiérrez, Epifanio Jiménez García, María Elena Sánchez Pardo. ENCB, Instituto Politécnico Nacional |

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| **M-31** | **Changed of platelet activity in patients with hypothyroidism**. *María Teresa Hernández Huerta***,** Laura Elena Pérez Ríos, Laura Pérez Campos Mayoral, Margarito Martínez Cruz, María del Socorro Pina Canseco, Eduardo Pérez Campos, Ruth Martínez Cruz. Unit of Biochemistry. ITO UNAM |
| **M-32** | **Effects of insulin and interleukin 10 (IL 10) in migration and proliferation process on HeLa, C33A and HaCat cells.** *Eva Hernández Márquez***,** Ivone Castro Romero, Anabel Martínez Baez, David Martínez Pastor, Elizabeth Sauceda Arellano, Diana Lashidua Fernández Coto, Ana Silvia Arenas Linares, Guadalupe Ayala Aguilar. CISEI. Instituto Nacional de la Salud Pública |
| **M-33** | **Antineoplastic capacity of *Moringa oleifera* extract through regulation of pyruvate dehydrogenase complex in a breast cancer murine model.** *Jessica Lizbeth Hernández Rivera,* Mara Ibeth Campos Almazán, Jaime Abraham de Lira Sánchez, Erick Sierra Campos, Mónica Andrea Valdéz Solana, Alfredo Téllez Valencia, Claudia Isela Avitia Domínguez. Facultad de Medicina y Nutrición. Universidad Juárez del Estado de Durango |
| **M-34** | **Thiamine deprivation produces similar metabolic and genomic effects as biotin deficiency**. *Alain Hernández Vázquez***,** Josué Andrés García Sánchez, Elizabeth Moreno Arriola, Ana Salvador Adriano, Daniel Ortega Cuéllar, Isabel Ibarra González, Antonio Velázquez Arellano. Unidad de Genética de la Nutrición. Instituto de Investigaciones Biomédicas, UNAM |
| **M-35** | **Alzheimer´sDisease: In TheEye?** *Luis Fernando Hernández Zimbron***,** Rubén ZamoraAlvarado, Lenin Ochoa de la Paz, Roberto González Salinas Edgar Zenteno Galindo, Hugo QuirozMercado. Unidad de Investigación, Asociación Para Evitar la Ceguera en México |
| **M-36** | ***In vitro* validation of differential gene expression for Acute Leukemia subtypification in Baja California Sur**. *Karina Elizabeth Jiménez Camacho***,** Reyna Romero Geraldo, Enrique Luna Taylor and Jesús González García. Instituto Tecnológico de la Paz |
| **M-37** | **Evaluation of the antifungal activity of imidazo[1,2-a]pyridine compounds on *Fusarium oxysporum* f. sp. *lycopersici*.** *Arturo Landeros de la Isla*, Paulina Serrano Sandoval, Manuel Velázquez Ponce, César Aza González, Karla Lizbeth Macías Sánchez. Instituto Politécnico Nacional |
| **M-38** | **Incidence Of Atherogenic Risk Factors In A Population Of The State Of Tabasco**. *Alejandra López Rivera*, José Joaquin Ovando Sánchez, Carlos Javier López Victorio. Universidad Juárez Autónoma de Tabasco |
| **M-39** | **Matrix Metalloproteinase (MMP)-28 increases proliferation rate and migration of lung alveolar and bronchial epithelial cells, and localizes in the nuclei of alveolar epithelial cells in Idiopathic Pulmonary Fibrosis.** *Mariel Maldonado,* Iliana Herrera, Ivette Buendía Roldán, Remedios Ramírez, Blanca Ortíz Quintero, Alfonso Salgado Aguayo, Moisés Selman, Annie Pardo. Facultad de Ciencias, UNAM |
| **M-40** | **Vasculogenic mimicry is inhibited by angiomiR miR-204 in breast cancer**. *Gilberto Mandujano Lázaro*, Ali Flores Pérez, Laurence A. Marchat, Sergio Rodríguez Cuevas, César López Camarillo. Posgrado en Ciencias Genómicas. Universidad Autónoma de la Ciudad de México |
| **M-41** | **Identification of oral bacteria associated with periodontal disease in patients on chronic hemodialysis**. *América Susana Mares García***,** Jesús Antonio Viana Rojas, Amaury Pozos Guillén, Juan Manuel López Quijano, Antonio Gordillo Moscoso. Facultad de Medicina. Universidad Autónoma de San Luis Potosí |
| **M-42** | **Comparison of commercial kits for molecular biology research**. *América Susana Mares García*. Diffractia México |
| **M-43** | **Early activation of autophagy in response to DNA damage caused by Irinotecan in mammal cells.** *Zaida Escila Martínez Moreno***,** Gabriel Muciño Hernández, Susana Castro Obregón. Departamento de Neurodesarrollo y Fisiología, División de Neurociencias.UNAM |
| **M-44** | **Omega-3 fatty acids and diabetes: effects on lipid metabolism and biological membranes.** *Ricardo Mejía Zepeda***,** Mónica Rivera Valencia, María del Consuelo Figueroa García. FES Iztacala, UNAM |
| **M-45** | **Combination treatment with ATRA and Fulvestrant inhibits mammary carcinoma cell migration and metastasis in a chicken embryo model**. *Rosa Isela Mendizabal Riveros***,** Omar Fernando Cortés Ponce De León, Axayacatl Morales Guadarrama, José Antonio Herrera Barragán, Claudia Haydée González Dela Rosa, Juan Gabriel Rivera Martínez, Javier Esteban Jiménez Salazar and Pablo Damián Matsumura. Biología de la Reproducción. Universidad Autónoma Metropolitana |

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| **M-46** | **Effect of matrix stiffness on normal human lung fibroblasts.** *Andrea Magali González Mora,* Mathieu Hautefeuille, Moises Selman, Annie Pardo. Laboratorio de Bioquímica, Departamento de Biología Celular, Facultad de Ciencias,UNAM |
| **M-47** | **Dysregulated calcium signals in tumoral cells MCF-7.** *Verónica Morales Tlalpan***,** Carlos Saldaña, Gustavo Acosta Santoyo, Aura Moreno Vega, Hebert Luis Hernández Montiel, Adriana Jheny Rodríguez Méndez, Mauricio Díaz Muñoz. Facultad de Ciencias Naturales. Universidad Autónoma de Querétaro |
| **M-48** | **Tepary bean lectins fraction (*Phaseolus acutifolius*) induces apoptosis in cell lines of colon cancer.** *Ulisses Moreno Celis***,** Roberto Augusto Ferríz Martínez, Adriana Jhenny Rodríguez Méndez, María del Carmen Mejía Vázquez, Alejandro Blanco Labra, Teresa García Gasca. Facultad de Ciencias Naturales. Universidad Autónoma de Querétaro |
| **M-49** | ***Stevia rebaudiana Bertoni* promotes insulin expression and release in Wistar rats fed with a hypercalorie diet.** *Diana Moroni González,* Patricia Aguilar Alonso, Eduardo Miguel Brambila Colombres, Alfonso Daniel Díaz Fonseca, Samuel Treviño Mora. Laboratorio de Investigaciones Químico Clínicas, Universidad Autónoma de Puebla |
| **M-50** | **Link between triglycerides, SCFAs levels and Colon microbiota functional metabolic profile in the mexican childhood obesity.** *Selvasankar Murugesan,* Otoniel Maya, Maria Luisa Pizano Zárate, Flor María Galván Rodríguez, Carolina Miranda Brito, Marta Romano Pardo, Alberto Piña Escobedo, Carlos Hoyo Vadillo, Jaime García Mena. Departamento de Genética y Biología Molecular, CINVESTAV Zacatenco |
| **M-51** | **Nutraceutical effects by peptides and metabolites from chia: the golden crop of the 21st century.** *Domancar Orona Tamayo***,** María Elena Valverde and Octavio Paredes López. CINVESTAV Irapuato |
| **M-52** | **Anti-mitochondrial therapy against triple negative breast cancer**. *Silvia Cecilia Pacheco Velázquez*, Rafael Moreno Sánchez, Sara Rodríguez Enríquez. Instituto Nacional de Cardiología "Ignacio Chávez" |
| **M-53** | **Nicorandil increases activity of complex I and III of the electron transport chain in atrophied muscle mitochondria**. *Padilla Maldonado Paulina***,** Sánchez Pérez Tania Alina, Sánchez Duarte Elizabeth, Cortés Rojo Christian, Saavedra Molina Alfredo, Montoya Pérez Rocío. Instituto de Investigaciones Químico Biológicas. Universidad Michoacana de San Nicolás de Hidalgo |
| **M-54** | **A novel panel of breast cancer-associated auto-antibodies in serum against recombinant tumor-associated antigens**. *Cecilia Pagaza Straffon***,** Laurence A. Marchat, José Díaz Chavez, Mauricio González Avante, Rosalba Carmona, Mauricio Castañon, Yadira Palacios, César López Camarillo. Posgrado en Ciencias Genómicas, Universidad Autónoma de la Ciudad de México |
| **M-55** | **Impact of type 1 diabetes on liver Acetyl CoA carboxylase of mice with different biotin Status.** *Cecilia Palmas Bustamante,* Laura P. Guerrero Carrillo, Ana L. Vázquez Jiménez, Miguel Anastacio Villanueva, Cecilia Ayala Zambrano, Jorge Omar García Rebollar, Georgina Díaz Herrera, Nancy Y. Mora Pérez, Armida Báez Saldaña. Instituto de Investigaciones Biomédicas, UNAM |
| **M-56** | **Effects of Pharmacological Concentration of Biotin on Testis and Expression levels of Acrogranin in Mice**. *Karina Pasten Hidalgo,* Gloria Sicilia Argumedo Cristina Fernández Mejía. Instituto de Investigaciones Biomédicas, Instituto Nacional de Pediatría |
| **M-57** | **TIGAR knockdown carry out to metabolic changes in cancer cell lines affecting survival**. *Miguel Ángel Peña Rico***,** María Nieves Calvo Vidal, Ruth Villalonga, Fina Martínez, Pepita Giménez, Aurea Navarro Sabaté, Avelina Tortosa, Ramón Bartrons and Anna Manzano. Bioquímica, Facultad de Medicina, UNAM |
| **M-58** | **Evaluation of the DNA integrity in isolated nuclei obtained from mouse spermatozoa.** *Rocío Quiroga Moreno***,** Rosalina Reyes Luna, Juan Carlos Flores Alonso. Centro de Investigación Biomédica de Oriente. IMSS |
| **M-59** | **Diabetes Mellitus type 2 decrease migratory capacity and store operated calcium entry in cardiac fibroblasts.** *Josué Miguel Julián Ramírez Reyes***,** Eduardo Monjaraz, Roberto Berra Romani, José Everardo Avelino Cruz. Facultad de Medicina, Universidad Autónoma de Puebla |
| **M-60** | **Isolation of an RNA aptamer population against HPV-16 L1 protein.** *Sergio Israel Rangel Guerrero***,** Luis Marat Álvarez Salas. Departamento de Genética y Biología Molecular.CINVESTAV Zacatenco |

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| **M-61** | **Serum iron is associated with dyslipidemia in a population of Mexico City.** *Susana Rivera Mancía***,** Maite Vallejo Allende, Eloísa Colín Ramírez, Raúl Cartas Rosado, Oscar Infante Vázquez, Jesús Vargas Barrón. CONACyT. Instituto Nacional de Cardiología "Ignacio Chávez" |
| **M-62** | **Synergistic evaluation of anti-mitochondrial and clinical drugs in cervix cancer growth.** *Diana Xochiquetzal Robledo Cadena***,** Sara Rodríguez Enríquez. Departamento de Bioquímica. Instituto Nacional de Cardiología "Ignacio Chávez" |
| **M-63** | **Biomarker profile of oncogenic proteins in patients with cardiac myxoma.** *Sara Rodríguez Enríquez***,** Silvia Cecilia Pacheco Velázquez, Juan Carlos Gallardo Pérez, Alhelí Adán Ladrón de Guevara, Jesús Vargas Barrón. Departamento de Bioquímica y Dirección de Investigación, Instituto Nacional de Cardiología "Ignacio Chávez" |
| **M-64** | **Differential effect of molecular iodine in mammosphere culture of breast cancer MCF-7 cells.** *Gabriel Miguel Rodríguez Gómez,* Evangelina Delgado, Carmen Aceves. Instituto de Neurobiología, UNAM |
| **M-65** | ***In vitro* identification of differential gene expression in patients with colorectal cancer in Baja California Sur.** *Geovanni Ruiz Romero,* Reyna Romero Geraldo Jesús Gonzales García, Mauricio Rodríguez Ojeda. Instituto Tecnológico de la Paz |
| **M-66** | **Proteomic study on mitochondrial complex IV disease using primary skin fibroblast cultures.** *Karina Olivia Salvador Severo*, Rosa María Ribas Aparicio, José García Trejo, Juan Fernando Minauro Sanmiguel. Hospital Pediatrico. CMN SXXI. IMSS |
| **M-67** | **Analysis of circulating microRNAs in plasma of gastric cancer patients.** *Mayra Cecilia Suárez Arriaga*, Torres López Javier, Camorlinga Ponce Margarita, Piña Sánchez Patricia, Valdez Salazar Hilda Alicia, Ribas Aparicio Rosa María, Fuentes Pananá Ezequiel M, Ruiz Tachiquín Martha Eugenia. Instituto Politécnico Nacional |
| **M-68** | **Identification of IgE binding proteins from *Ligustrum lucidum* using an Immunoproteomics approach.** *Luis Manuel Terán Juárez***,** Blessy Maruthukunnel Mani, Erik Elvin Gómez Cardona, Gandhi Fernando Pavón Romero, Alberto Barrera Pacheco, Ana Paulina Barba de la Rosa. Instituto Nacional de Enfermedades Respiratorias "Ismael Cosio Villegas" |
| **M-69** | **Effect of biotin supplementation during different stages of pancreatic development.** *WilmaTixi Verdugo***,** Juan Contreras Ramos, Gloria Sicilia Argumedo, Cristina Fernández Mejía. Unidad de Genética de la Nutrición, Instituto de Investigaciones Biomédicas, UNAM |
| **M-70** | **Early differential immuneregulation in acute lung injury induced by systemic inflammatory response syndrome or sepsis.** *Janette Arias Escobedo*, Sonia Ivonne Rodríguez Ruiz, Faber Ignacio García Lorenzo, Marco Antonio Checa Caratachea, Joaquin Alejandro Zuñiga Ramos, Edgar Zenteno Galindo, Francisco Javier Urrea Ramírez. Departamento de Bioquímica, Instituto Nacional de Enfermedades Respiratorias Ismael Cosio Villegas |
| **M-71** | **Cardiovascular risk factors defined by the global scale Framingham in a group of adults residing in Mexico City**. *Vera Rosales María del Carmen,* Aguilar Sánchez Mariana, Huerta Huerta Raquel, Núñez Cardona Ma. Teresa. Universidad Autónoma Metropolitana. Unidad Xochimilco |
| **M-72** | **Relationship between total cholesterol and triglycerides levels with body mass index and dietary ingest in a group of workers.** *Vera Rosales Ma. Del Carmen*, López Flores Alejandra, Trejo Sánchez Hazael, Huerta Huerta Raquel, Núñez Cardona Ma. Teresa. Universidad Autónoma Metropolitana |
| **M-73** | **HIV: discrimination and stance young university people infected.** *Olga Selene Vidal Lucas*, Blanca Estela Trejo Sánchez, Lucas López Segovia. Universidad Juárez Autónoma de Tabasco |
| **M-74** | **Comparative side effects of two inhibitors of the 3-Hydroxy-3-Methylglutaryl Coenzyme-A Reductase: atorvastatin and rosuvastatin, when administered in high doses to rodents with a choresterol rich diet.** *Alma Mileira Zetina Esquivel***,** Isela Esther Juárez Rojo, Andrés Eliud Castell Rodríguez, José Luis Blé Castillo, Ligia Araceli Barragán Lizama, Rodrigo Miranda Zamora, Juan Cuauhtémoc Díaz Zagoya. Ciencias de la Salud. Universidad Juárez Autónoma de Tabasco |
| **M-75** | **Antioxidant capacity and physicochemical characteristics of wine from jamaica (*Hibiscus sabdariffa*, L.)** *Julia Verónica Hernández Madrigal*, Laura Virginia Madrigal Ambriz, Joel Vazquez Galindo. Facultad de Ciencias Químicas. Universidad de Colima |

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| **M-76** | **Proteomic analysis of exosomes from serum of breast cancer women and citokines-stimulated cell lines**. *Felipe de Jesús Palacios Castañeda*, Israel Romo Cruz, Gloria León Ávila, Juan Pedro Luna Arias, Armando Pérez Rangel, José Manuel Hernández Hernández. CINVESTAV Zacatenco |
| **M-77** | **Leishmanicidal drug design. Induce fit studies to find potential inhibitors against arginase from *Leishmania Mexicana***. *María Irene Betancourt Conde*, Alondra Chaidez Avila, Alejandra Guadalupe Vázquez Raygoza, José Luis Urbán Martínez, Daniel Enríquez Mendiola, Claudia Isela Avitia Domínguez, Antonio Romo Mancillas, Alicia Hernández Campos, Alfredo Téllez Valencia. Facultad de Medicina, Universidad Juárez del Estado de Durango |

# *Wednesday*

Wednesday November 9, 2016

**8:30 – 10:30 Plenary Lectures**

8:30 – 9:30 Influenza virus-host interactions
**Adolfo García Sastre**
Icahn School of Medicine at Mount Sinai. New York, NY. USA

Chair: *Jorge Luis Folch*
Universidad Autónoma del Estado de Morelos

9:30 – 10:30 mTOR and Regulation of Cell Growth
**John Blenis**
Sandra and Edward Cancer Center. Weill Cornell Medicine. USA

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

10:30 – 11:00 Coffee break
Foyer San Marcos Room

11:00 – 13:30 Technical Conferences
San Marcos Room IV

Chair: *Jorge Luis Folch Mallol*

11:00 – 11:30 UNIPARTS
**Cultivo Celular 3D**
*Javier Hernández Juárez*

11:30 – 12:00 GE HEALTHCARE
**Usando Análisis Celular Multiparamétrico por microscopia de fluorescencia (HCA) y microscopia de Alta y Súper resolución para resolver preguntas biológicas**
*Sandra Rosa Silva*

12:00 – 12:30 ACCESOLAB
**Acclaro Sample Intelligence technology: how it works and how sample purity is important for downstream applications**
*Voula Kodoyianni*. Thermo Fisher

12:30 – 13:00 DIFRACCTIA
**Mini PCR: Abriendo las puertas al análisis de ADN**
*Hugo Arellano Santoyo*
Harvard Medical School. Dana Farber Cancer Institute.
Howard Hughes Medical Institute

13:00 – 13:30 BIORAD
**Producción rápida de RNA quiméricos mediante cromatografía semipreparativa**
*Adriana Vega Belmont*

**Concurrent Sessions**

Wednesday November 9, 2016

| 11:00 – 13:30 | **7 Immunology & Parasitology**<br>**San Marcos One Room** | **8 Microbiology & Viruses**<br>**San Marcos Two Room** | **9 Signal Transduction & Cell Differentiation**<br>**San Marcos Three Room** |
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| | Chair: *Mónica Valdez*.<br>Univ. Juárez Edo. Durango | Chair: *Hortensia Silva*.<br>UABC | Chair: *Héctor Riveros*.<br>Facultad de Medicina, UNAM |
| 11:00-11:20 | **Identification of potential pathogenic microorganisms in the tract of nymphs and canaries in captivity.** *Mónica A. Valdez Solana*, Ulrik H. Pedroza Dávila, Ariel Rodarte Ramírez, Cristina García De La Peña, Erick Sierra Campos. Facultad de Ciencias Químicas, Universidad Juárez del Estado de Durango | **Dynamics of the actin cytoskeleton and vesicle traffic in the lack of MYO-5 in *Neurospora crassa*** *Arianne Ramírez del Villar*, Olga A. Callejas Negrete, Meritxell Riquelme, Rosa R. Mouriño Pérez. Centro de Investigación Científica y de Educación Superior de Ensenada | **Distinct phosphorylation patterns regulate α1D–adrenoceptor signaling and desensitization** *Marco A. Alfonzo Méndez*, Gabriel Carmona Rosas, Aurelio Hernández Méndez, David A. Hernández Espinosa, Ma. Teresa Romero Ávila, J. Adolfo García Sáinz. Instituto de Fisiología Celular, UNAM |
| 11:20-11:40 | **Fate of the host proteins internalized by cysticerci of *Taenia crassiceps.*** *Jeanette Flores Bautista*, José Navarrete Perea and Juan Pedro Laclette. Instituto de Investigaciones Biomédicas, UNAM | **Characterization of human papillomavirus type 33 variants circulating in San Luis Potosi, Mexico** *Ricardo Uribe Rodríguez*, Raúl De la Rosa Martínez, Víctor Sanabria Ayala, Mireya Sánchez Garza, Mariana Azanza Rodríguez, Rubén López Revilla. IPICYT | **Natural variation of the mutational effects of the Tor Pathway on yeast aging** *J Abraham Avelar Rivas*, Alejandro Juárez Reyes Alexander de Luna. LANGEBIO. CINVESTAV Irapuato |
| 11:40-12:00 | **Defensin γ-Thionin from *Capsicum chinense* has Immunomodulatory Effects on Bovine Mammary Epithelial Cells during *Staphylococcus aureus* Internalization.** *Ricardo Iván Medina Estrada*, Joel Edmundo López Meza, Alejandra Ochoa Zarzosa. Universidad Michoacana | **Novel microbial species isolated from subaerial biofilms of stone monuments** *Juan Vázquez Martínez*, Juan Manuel Gutiérrez Villagomez, Génesis V. Buitimea Cantúa, Enrique Ramírez Chávez, Jorge Molina Torres. CINVESTAV Irapuato | **Damage-associated molecular patterns (DAMPs) and signalling during mechanical injury in filamentous fungi** *Elizabeth Medina Castellanos*, Meritxell Riquelme, Nick D. Read y Alfredo Herrera Estrella. National Laboratory of Genomics for Biodiversity |
| 12:00-12:20 | **Assessment of the antitumor and immunostimulatory activity of a commercial poultry vaccine.** *Oscar Antonio Ortega Rivera*, Schillberg Stefan, Raven Nicole, Del Toro Arreola Susana, Quintanar Stephano José Luis, Salinas Miralles Eva María. Universidad Autónoma de Aguascalientes | **Killer peptide targeting arrested cells.** *Vladimir Juárez Arellano*, Gabriel del Rio. Instituto de Fisiología Celular, UNAM | **Regulation of snon expression by TGFβ and GPCR signals in Hepatocytes** *Diana Grisel Ríos López*, Marina Macías Silva. Instituto de Fisiología Celular, UNAM |

| | 7 Immunology & Parasitology<br>San Marcos One Room | 8 Microbiology & Viruses<br><br>San Marcos Two Room | 9 Signal Transduction & Cell Differentiation<br>San Marcos Three Room |
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| | Chair: *Mónica Valdez* Solana Univ. Juárez Edo. Durango | Chair: *Hortensia Silva* UABC | Chair: *Héctor Riveros* Facultad de Medicina, UNAM |
| 12:20-12:40 | **Telomere repeat binding factors in expression of ehtrf-like proteins in H2O2 treated trophozoites of *Entamoeba histolytica.*** *Elisa Azuara Liceaga*, Vanessa Díaz Helios, Cárdenas Hernández David, Hernández Álvarez Elizabeth, J Castañeda Ortíz, Jesús Valdés Flores, José Manuel Galindo Rosales Guillermina García Rivera, Esther Orozco, Bibiana Chávez Muguia, Abigail Betanzos Fernandez, Jesús Valdés Flores, Francisco J Rendón Gandarilla. UACM | **Autophagy and senescence during the development of spinal cord and the differentiation of motoneurons.** *Jorge Antolio Domínguez Bautista*, Susana Castro Obregón. Instituto de Fisiología Celular, UNAM | **Molecular and biological characterization of TcVps26-Like of *Trypanosoma cruzi.*** *Margarita Rubio Ortiz*, Santiago Martínez Calvillo y Rebeca Georgina Manning Cela. CINVESTAV-IPN |
| 12:40-13:00 | **Structural characterization and cellular localization of Tv-PSP1, a perchloric acid-soluble protein of *Trichomonas vaginalis.*** *Alma Villalobos Osnaya*, Georgina Garza Ramos, César Millán Pacheco, Iris N. Serratos, Arturo González Robles, Rossana Arroyo and María Elizabeth Álvarez Sánchez. UACM | **Defective autophagy in Neuronal and Glial Senescence .** *Daniel Moreno Blas*, Elisa G. Gorostieta Salas, Gabriel Muciño Hernández, Alexander M. Pommer Alba y Susana Castro Obregón. Instituto de Fisiología Celular, UNAM | **Histamine Impairs Midbrain Dopamine Neuron Differentiation in Association to Modifications of Epigenetic DNA Marks.** *Fernanda Vargas Romero*, Ernesto Soto Reyes, Rodrigo González Barrios, Lissania Guerra Calderas, Iván Velasco.Instituto de Fisiología Celular-Neurociencias, UNAM |
| 13:00-13:20 | **Analysis of the adaptive immune response in mice with Chagas' disease treated with NIPOx-B.** *Carlos Wong Baeza*, Claudia Albany Reséndiz Mora, Carla Elizabeth Landa Saldívar, Oscar Arturo Ángeles Manzano, Mayra Atzin Hernández León, María Isabel Baeza Ramírez, Carlos Wong Ramírez. Instituto Politécnico Nacional | **Molecular analysis of the process of sarcopenia: Changes in muscle gene expression between young and older adults.** *Miguel Ángel Fonseca Sánchez*, Yanelli Trujillo Cabrera, José de Jesús Rivera Sánchez,Gerardo Aristi Alberto Flores Luce, Gloria Queipo García. Servicio de Medicina Genética, Hospital General de México Eduardo Liceaga | **Infected cells in root bean symbiotic nodules: an environment under controlled stress.** *Alejandra Zayas Del Moral*, Georgina Estrada Navarrete, Xóchitl Alvarado Affantranger, Damián Martínez Reyes, Carmen Quinto and Federico Sánchez. Instituto de Biotecnología, UNAM |

13:30 – 15:30 Lunch

Free Time

# *Thursday*

**8:30 – 10:30 Plenary Lectures**

8:30 – 9:30 The control of eukaryotic protein secretion by a novel pathway regulating glutathione traffic in and out of the endoplasmic reticulum

**Michel Toledano**
CEA Sciences Division. Institute of Biology and Technology Saclay. Integrative biology and molecular genetics unit. Oxidative Stress and Cancer Laboratory

Chair: *Alejandro de las Peñas*
IPICYT

9:30 – 10:30 Mitochondrial bioenergetics and OXPHOS supercomplexes

**Oscar Flores Herrera**
Departamento de Bioquímica. Facultad de Medicina, UNAM

Chair: *Federico Martínez Montes*
Facultad de Medicina, UNAM

10:30 – 11:00 Coffee break
Foyer San Marcos Room

**Concurrent Sessions**

Thursday November 10, 2016

| 11:00 – 13:30 | **10 Systems Biology & Bioinformatic**<br><br>**San Marcos One Room** | **11 Biotechnology**<br><br>**San Marcos Two Room** | **12 Genetic, Genetic Regulation & Epigenetic**<br><br>**San Marcos Three Room** |
|---|---|---|---|
| | Chair: *Gabriel del Rio*<br>I.Fisiología Celular, UNAM | Chair: *Ramón Alberto Batista*<br>Universidad Aut. Morelos | Chair: *Martha Calahorra*<br>I. Fisiología Celular, UNAM |
| 11:00-11:20 | **Predicting multifunctional polypeptides using machine learning algorithms**<br>*David Alejandro Castillo González*, Gabriel Del Río Guerra. Instituto de Fisiología Celular, UNAM | **Design of Multi-domain Proteins for Artificial Virus-like Supramolecular Nanoparticles**<br>*Armando Hernández García*, Daniela Kraft, Paul Van Der Schoot and Renko de Vries. Nortwestern University. Wageningen University | **Genome-wide profiling of DNA methylation in response to resveratrol identified novel therapeutic epigenetic targets in breast cancer cells.** *Rubiceli Medina Aguilar*, Carlos Pérez Plasencia, Patricio Gariglio, Jaime García Mena, José Ali Flores Pérez, Laurence A. Marchat, César López Camarillo. CINVESTAV IPN |
| 11:20-11:40 | **Finishing and validation of a lager beer yeast genome sequence using a BAC-based physical map, an hybrid approach**<br>*Cintia Noemí Gómez Muñoz*, Riego Ruiz Lina, Montalvo Arredondo Javier, Pérez Ortega Esmeralda and Damas Buenrostro Luis. IPICyT | **Experimental strategies to enhance activity and stability of functional laccase from *Therrmus thermophilus***<br>*Beatriz Miranda Zaragoza*, Paul Gaytán, Horacio Reyes Vivas, Enrique Rudiño Piñera, Claudia Rodríguez Almazán. Instituto de Biotecnología, UNAM | **Establishment of molecular basis of the classification of histone deacetylase**<br>*Jazmín Eliana Murcia Garzón*, Gustavo Hernández Guzmán, Varinia López Ramírez, Juan Manuel González Prieto. Centro de Biotecnología Genómica, IPN |
| 11:40-12:00 | **Inter-chromosomal Transcriptional Regulation in Breast Cancer**<br>*Jesús Espinal Enríquez*, Cristóbal Fresno, Guillermo de Anda, Enrique Hernández Lemus. Instituto Nacional de Medicina Genómica | **PH effect on growth and lipids accumulation in *Chlamydomonas reinhardtii***<br>*Ana Erika Ochoa Alfaro*, Daniel Eugenio Gaytán Luna, Alejandro Rocha Uribe, Ruth Elena Soria Guerra. Facultad de Química, Universidad Autónoma de San Luis Potosí | **Expression pattern of miRNAs associated with the response to conventional treatment in patients with cervical cancer**<br>*Abraham Pedroza Torres*, Oliver Millán Catalán, Yahir Alberto Loissell Baltazar, Carlos Pérez Plasencia. Instituto Nacional de Cancerología |
| 12:00-12:20 | **Visualization of F-actin *Trichoderma atroviride* during growth and mechanical injury**<br>*Marisela Garduño Rosales,* Elizabeth Medina Castellanos, Olga A. Callejas Negrete, Alfredo Herrera Estrella and Rosa R. Mouriño Pérez. Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada | **Padlock probe-rolling circle amplification assays for the detection of high risk human papilloma viruses**<br>*Lucía Orellana Escobedo*, Cindy Gómez Correa, Mireya Sánchez Garza, Rubén López Revilla. Departamento de Biología Celular, IPICYT | **DNA methylation profiling at a single locus of GNPDA2, PPARGC1α and LEPR genes from umbilical cord and the correlation analysis to maternal-fetal anthropometry in pregnancy.** *Erika Chavira Suárez***,** Jorge Beltrán Montoya, Carmen Canchola Sotelo, Felipe Vadillo Ortega. Unidad de Vinculación Científica de la Facultad de Medicina, UNAM |

| | **10 Systems Biology & Bioinformatic**<br><br>**San Marcos One Room** | **11 Biotechnology**<br><br>**San Marcos Two Room** | **12 Genetic, Genetic Regulation & Epigenetic**<br><br>**San Marcos Three Room** |
|---|---|---|---|
| | Chair: *Gabriel del Rio*<br>I. Fisiología Celular, UNAM | Chair: *Ramón Alberto Batista*<br>Universidad Aut. Morelos | Chair: *Martha Calahorra*<br>I. Fisiología Celular, UNAM |
| 12:20-12:40 | **Pore-forming mechanism of the antimicrobial peptide Pandinin-2.**<br>*Ramón Garduño Juárez*, José Luis Velasco Bolom. Instituto de Ciencias Físicas, UNAM | **Exploring PD-1 and PD-l1 conformational landscape using molecular dynamics simulations.**<br>*Marcelino Arciniega Castro*. Instituto de Fisiología Celular, UNAM. | **An adenosine derivative compound exerts a hepatoprotective effect involving epigenetic changes in CCl4 – induced rat cirrhosis.**<br>*Jesús Rafael Rodríguez Aguilera*, Carlos Alberto Guerrero Hernández, Rosario Pérez Molina, Carla Elizabeth Cadena del Castillo, Rebeca Pérez Cabeza de Vaca, Nuria Guerrero Celis, Adrián Rafael Murillo de Ozores, Brenda Elizabeth Miranda Hernández, Félix Recillas Targa, Victoria Chagoya de Sánchez. Instituto de Fisiología Celular, UNAM |
| 12:40-13:00 | **Molecular signatures of mitochondrial metabolism shift in glioblastoma cell lines defined by principal component analysis.**<br>*Leopoldo Gómez Caudillo,* Fernando Minauro Sanmiguel, Ariadna Jazmín Ortega Lozano, Haydeé Rosas Vargas, Sergio Manuel Encarnación Guevara.Unidad de Investigación Médica en Genética Humana, UMAE Pediatría CMNSXXI | **Induction of IL-10 and TGF-β in intestinal mucosa of rat by *lactococcus lactis* IL-22 secretor. An alternative treatment of experimental liver cirrhosis.**<br>*Alonso Prieto Javier*, Michelle Andrea Delgado García, Martin Humberto Muñoz Ortega, Daniel Cervantes García, Odila Saucedo Cárdenas, Roberto Montes de Oca Luna, María de Jesús Loera Arias, Javier Ventura Juárez. Universidad Autónoma de Aguascalientes | **Profile of cytokine/ chemokines associated to Th17 response as distal progression markers in patients with Locally Advanced Cervical Cancer (LACC).**<br>*Horacio Zamudio Meza*, Jorge Fernández Retana, Miguel Rodríguez Morales, Jaime Coronel Martínez, David Francisco Cantú de León, Carlos Pérez Plasencia. Instituto Nacional de Cancerología |
| 13:00-13:20 | **Towards the Inference of the Atomic Three-Dimensional Structural Models of Proteins Without Crystallography nor Resonance.**<br>*Gabriel Del Rio Guerra*, María Teresa Lara, Ricardo Corral, Víctor Martinell. Instituto de Fisiología Celular, UNAM | **Effect of aging in lungs of normal and bleomycin-induced fibrosis analyzed in wild type and accelerated aging *Zmpste24* deficient mice.** *María del Jazmín Calyeca Gómez*, Olmos Raúl, Jasso Rogelio, Gaxiola Miguel, LópezOtín Carlos, Selman Moisés and Pardo Annie. Facultad de Ciencias, UNAM | **Cytotoxic Effects of Avocado Lipids (*Persea americana* var. drymifolia) on cancer cells.**<br>*Mónica Lara Márquez*, Marisol Báez Magaña, Patricia Nayeli Alva Murillo, Rafael Salgado Garciglia, Alejandra Ochoa Zarzosa y Joel Edmundo López Meza. FMVZ. Universidad Michoacana de San Nicolás de Hidalgo |

13:30 – 15:30 Lunch

**Plenary Session 7**
Thursday November 10, 2016
15:30 – 18:00 San Marcos Room I

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**Oxidative Stress in Life**

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

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15:30 – 16:00 Positive effects of (-)-epicatechin and epicatechin rich cocoa on indicators of mitochondrial biogenesis, oxidative stress in senile mice and in patients with type 2 diabetes
**Guillermo Ceballos**
Laboratorio Investigación integral cardiometabólicas. Sección de Posgrado, Escuela Superior de Medicina. IPN

16:00 – 16:30 Visualization of reactive oxygen species dynamics in developing zebrafish embryos
**Enrique Salas Vidal,** Mario Adán Mendieta Serrano, Francisco Javier Méndez Cruz, Luis Cárdenas, Mayra Antúnez Mojica, Laura Álvarez, Denhi Schanbel Peraza, Hilda Lomelí
Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología. UNAM

16:30 – 17:00 The several faces of the P2X7 receptor: a cation cannel that triggers reactive oxygen species production, cell death and interleukin production
**Jorge Arreola**
Physics Institute, Universidad Autónoma de San Luis Potosí

17:00 – 17:30 Reactive oxygen species as key regulators of polar growth and symbiosis
**Luis Cárdenas**, Fernando Lara, Ramsés García Niño, Alejandra Hernández Barrera, Ana VelardeBuendía, Rocío Pérez, Rosana Sánchez, Johnson E, Heng Ming Whu, Federico Sánchez, Carmen Quinto, Alice Cheung
Instituto de Biotecnología, UNAM

**Plenary Session 8**
Thursday November 10, 2016
15:30 – 18:00 San Marcos Room II

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**Bioenergetic**

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

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15:30 – 16:00 Peroxisome and mitochondrial dynamics during sexual development of the fungus *Podospora anserina*
**Leonardo Peraza Reyes**, Jorge Luis CastilloCanizáles, Harumi Takano-Rojas y Fernando Suaste Olmos
Instituto de Fisiología Celular, UNAM

16:00 – 16:30 Mitochondrial proteomic analysis during development of chemically induced hepatocellular carcinoma in rats: an evolutionary perspective
**Rafael Montiel**, J. Noé GarcíaChávez, Hilda E. RamosAboites, Verónica R. Vázquez Garzón, Christian E. Martínez Guerrero, Christian LonaArrona, Saúl VillaTreviño
LANGEBIO CINVESTAV Irapuato

16:30 – 17:00 Anti-mitochondrial therapy against malignant tumors
**Sara Rodríguez Enríquez** and Rafael Moreno-Sánchez
Departamento de Bioquímica, Instituto Nacional de Cardiología

17:00 – 17:30 Systematic identification of cellular-aging factors and dietary-restriction effects
**Alexander de Luna**, Sergio E. Campos, Erika Garay & Alejandro Juárez-Reyes
LANGEBIO CINVESTAV Irapuato

**Plenary Session 9**
Thursday November 10, 2016
15:30 – 18:00 San Marcos Room III

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**Emerging Viruses**

Chair: *Carlos Arias Ortiz*
Instituto de Biotecnología, UNAM

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15:30 – 16:00 Environmental and biological drivers for viral infection distribution and disease occurrence
**Gerardo Suzán**
Facultad de Medicina Veterinaria y Zootecnia. UNAM

16:00 – 16:30 *Aedes aegypti* immune response against dengue virus and other pathogens
**Humberto Lanz Mendoza**, Salvador Hernández Martínez
Center for Research on Infectious Diseases, National Institute of Public Health

16:30 – 17:00 Cross reactivity between dengue virus and zika viruses: consequences for ZIKA virus pathogenesis and prevention
**Juan Ernesto Ludert**
Departamento de Infectómica y Patogénesis Molecular. CINVESTAV

17:00 – 17:30 The ZIKA virus: epidemiology and pathogenesis
**Rosa María del Angel**
Departamento de Infectómica y Patogénesis Molecular. CINVESTAV

18:00 – 20:00 Poster Session 3

GR GENETIC, EPIGENETIC AND GENETIC REGULATION
MV MICROBIOLOGY AND VIRUSES

## GENETIC, EPIGENETIC AND GENETIC REGULATION

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| **GR-1** | **Association of single nucleotide polymorphisms of the APLN, APLNR and MTHFR genes with the presence of essential hypertension in postmenopausal Yucatecan mestizo women.** *Jennifer Alcántara Blancarte*, Thelma Canto Cetina, Javier Cano, Rosa Esteban, Sergio De los Santos, Patricia Canto, Ramón Mauricio Coral Vázquez. Facultad de Medicina, UNAM |
| **GR-2** | **Phenotypic characterization of the maize *med12* mutants.** *Ana Laura Alonso Nieves***,** Tania Núñez Ríos, Carol Martínez Camacho, Stewart Gillmor, Ruairidh Sawers. LANGEBIO, CINVESTAV Irapuato |
| **GR-3** | **Identification of Lectin from teosinte coleoptile (*Zeadiploperennis*).** *Jaquelina Alvarado Gil***,** Carlos Alberto Matías Cervantes, Nora Hilda Rosas Murrieta, Eduardo Pérez Campos and Margarito Martínez Cruz. Instituto Tecnológico de Oaxaca |
| **GR-4** | **Evaluation of CDKN3 in cell cycle involvement in cell lines derived from cervical cancer.** *Erika Anabel Alvarado Silva***,** Eira Valeria Barrón Palma, Maura Guadalupe Bautista Huerta, América Gutiérrez Castro, Angel Tonatiuh Salazar Anzures, Jaime Berumen Campos. Unidad de Medicina Genómica, Hospital General de México "Dr. Eduardo Liceaga" |
| **GR-5** | **Nuclear RNA extraction as a way to study lncRNAs in *Ustilago Maydis.*** *Estela Anastacio Marcelino***,** Erick Accocatl Juárez, Antonio Sampedro Luna, Candelario Vázquez Cruz, Ma. Patricia Sánchez Alonso. Instituto de Ciencias, Universidad Autónoma de Puebla |
| **GR-6** | **Tumor suppressor miR-29c regulates radioresistance in lung cancer cells.** *Elena Aréchaga Ocampo***,** Claudia H. Gonzalez De la Rosa, Isidro X. Perez Añorve, Reynalda Roldan Perez, Ali Flores Perez, Omar Peña Curiel, Oscar Ángeles Zaragoza, Nohra E. Beltran Vargas, Rosalva Rangel Corona, Nicolas Villegas Sepulveda. Universidad Autónoma Metropolitana Unidad Cuajimalpa |
| **GR-7** | **Functional subfunctionalization of branched chain amino transferases through diversification of transcriptional regulators and chromatin organization in the yeast *Saccharomyces cerevisiae.*** *Stefany Argueta Zepeda***,** James González, Geovani López, Dariel Márquez, Carlos Campero Basaldua, Joseph Strauss, Lina Riego Ruiz and Alicia González. Instituto de Fisiología Celular, UNAM |
| **GR-8** | **Genomic and functional studies of miRNA regulation of Arabidopsis embryogenesis**. *Alma Armenta Medina***,** R. Scott Poethig and Stewart Gillmor. LANGEBIO. CINVESTAV Irapuato |
| **GR-9** | **Association of *VDR, KLOTHO, CYP27B1, PTPN22* and *AGTR2* SNPs with clinical characteristics in turner syndrome patients.** *Rehotbevely Barrientos*, Leda Torres, José Velázquez, Camilo Villarroel, Silvia Sanchez, Nelly Altamirano, Sara Frias. Instituto Nacional de Pediatría |
| **GR-10** | **MiR34a as possible regulator of ubiquitin ligase E6AP in a model of HPV18 E6 overexpression.** *Bautista Isidro Luis Osvaldo***,** García Castillo Verónica, López Urrutia Eduardo, Pérez Plasencia Carlos.Unidad de Biomedicina Facultad de Estudios Superiores Iztacala, UNAM |
| **GR-11** | **Risk factors associated with lack of weight reduction in morbidly obese patients.** *Tania Berenice Bazán Soto***,** Andy Michel Romero Nieves, Etual Espinosa, Rodolfo Rivas Ruiz, Claudia Ramírez Rentería, Mario Molina Ayala, Moisés Mercado, Osvaldo Daniel Castelán Martínez. FES Zaragoza, UNAM |
| **GR-12** | **Gene expression profile of HSP90AA1 y HSP90AB1 in clear renal cell carcinona (CRCC) and clinical implications**. *Brenda Larissa Calvo Hernández,* Nadia Aglae Rangel Gauna, Gpe. Lizeth Gutiérrez Murguía, María Guadalupe Gracia Muñoz, Luis Alonso Herrera Montalvo, Carlo César Cortés González. Instituto Nacional de Cancerología |
| **GR-13** | **Expression profile of microRNAs in small cell lung cancer tumors.** *María de los Ángeles Carlos Reyes,* J Sullivan Lopez Gonzalez, Nayeli Ramírez Torres, Cesar Rivero Luna, Concepción Ortega Carrillo, Raúl García Vazquez, Raquel Echeverria Zepeda, Carlos Palma Flores, Ali Flores Pérez and Cesar Lopez Camarillo. Instituto Nacional de Enfermedades Respiratorias Ismael Cosio Villegas |
| **GR-14** | **Evaluation of gamma and delta tubulins in cervical cancer progression.** *Ricardo Castillo Velazquez***,** Susana López Robles, Claudia Araceli Reyes Estrada, Rosalinda Gutiérrez Hernández and Jesús Adrián López. Universidad Autónoma de Zacatecas "Francisco García Salinas" |
| **GR-15** | **A *Phaseolus vulgaris* annexin modulates the rhizobial infection and the nodulation process.** *Janet Carrasco Castilla***,** Yolanda Ortega Ortega, David Jáuregui, Marco A. Juárez Verdayes, Rosana Sánchez López, Elizabeth Monroy, Noreide Nava, Olivia Santana, Carmen Quinto. Instituto de Biotecnología. UNAM |

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| **GR-16** | **Regulator ThnR and ABC transporter are necessary to immunity of *Bacillus thuringiensis* against Thurincin H(m).** *Luz Edith Casados Vázquez,* José Eleazar Barboza Corona. Universidad de Guanajuato Campus Salamanca |
| **GR-17** | **Analysis of expression of TFIIB1 transcription factor in *Arabidopsis thaliana* and *Phaseolus vulgaris*.** *Saraí Castro Bustos***,** Augusto Ramírez Trujillo, Nancy Sofía Hernández Bueno, Víctor Manuel Bustos Zagal, Mario Ramírez Yáñez, Ramón Suárez Rodríguez. Centro de Investigación en Biotecnología. Autónoma del Estado de Morelos |
| **GR-18** | **Preliminary study about the effect of maternal obesity on global DNA methylation in placenta.** *Violeta Castro Leyva***,** Felipe Vadillo Ortega, Maria del Carmen Garcia de Leon Mendez, Erika Chavira Suarez. Departamento de Bioquímica, Facultad de Medicina, UNAM |
| **GR-19** | **Bariatric surgery-associated DNA methylation remodeling, in adipose tissue from obese patients.** *Federico Centeno Cruz***,** Ernesto Sánchez, Guillermo Garduño, Paulina Baca Peynado, Angélica Martínez, Carlos Zerrweck, Francisco Barajas, Lorena Orozco. Laboratorio de Inmunogenómica y Enfermedades Metabólicas. Instituto Nacional de Medicina Genómica |
| **GR-20** | **Transcriptomic analysis of the adaptation of *Ustilago maydis* to changes of pH.** *Juan Antonio Cervantes Montelongo,* Elva Teresa Aréchiga Carvajal, José Ruiz Herrera. CINVESTAV Irapuato |
| **GR-21** | **The role of dAtrx ATPase in the telomeric maintenance of *Drosophila melanogaster***. *Joselyn Cristina Chávez Fuentes***,** Vanessa Bahena Villamil, Juan Manuel Murillo, América Castañeda Sorbitrán, Rosario Rodriguez Arnaiz, Mario Zurita Ortega, Viviana Valadez Graham. Departamento de Genética del Desarrollo y Fisiología Molecular. Instituto de Biotecnología, UNAM |
| **GR-22** | **Ribosomal protein S1 is required for recognition of downstream adenine- or uracil-rich mRNAs by 30S subunit.** *Juan Carlos Cifuentes Goches***,** Olvera Maturano Nubia Jazmín, Salinas Tobón María del Rosario, Guarneros Gabriel, Hernández Sánchez Javier. CINVESTAV Zacatenco |
| **GR-23** | **Analysis of the regulation exerted by mir-26a on the *rb1* and *apc* messengers associated to colorectal cancer**. *Carlos Contreras Romero***,** E. Lopez Urrutia, V. García Castillo, C. Pérez Plasencia. FES Iztacala, UNAM |
| **GR 24** | **The strands 5p and 3p of the members of the family miR-34 have differential effects on SiHa cell proliferation and migration.** *Sergio Cordova Rivas***,** José Luis Ruiz Carrillo, Ruth Cristina Rodríguez Garces, Hiram Hernández López, Jesús Adrián López. Universidad Autónoma de Zacatecas |
| **GR 25** | **mir- 26a, epigenetic regulator associated with colorectal cancer.** *Coronel Hernández Jossimar***,** García Castillo Verónica, López Urrutia Eduardo, Pérez Plasencia Carlos. FES Iztacala, UNAM |
| **GR-26** | **The role of Tmk3-Atf1 pathway on blue light responses in *Trichoderma atroviride*.** *Víctor Alejandro Correa Pérez***,** Roberto Álvarez Martínez, Fidel Landeros Jaime, José Antonio Cervantes Chávez, Alfredo Herrera Estrella, Edgardo Ulises Esquivel Naranjo. Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro |
| **GR-27** | **Assessing the level of expression of mir 132 and 203, and the methylation status of the promoter region of this miRNAs in cell lines of breast cancer and their role as posttranscriptional regulators repair genes DNA damage BRCA-1, BRCA -2 and ATM.** *Pablo Cortés Pérez***,** López Urrutia Eduardo, García Castillo Verónica, Pérez Plasencia Carlos. FES Iztacala, UNAM |
| **GR-28** | **Hyperglycemic effect on proliferation and apoptosis of human umbilical cord mesenchymal stem cells from children of normoglycemic and diabetic mothers.** *Sandra Rocío Cruz Bárcenas***,** Josiff S. Flores Reyes, Oscar Pérez Pérez, Ismael Mancilla Herrera, Mauricio Domínguez Castro, Higinio Estrada Juárez, Enrique Reyes Muñoz, Mónica Aguinaga Rios, Patricia Grether González, José Romo Yáñez. Facultad de Medicina, Instituto Nacional de Perinatologia "Isidro Espinosa de los Reyes" |
| **GR-29** | **Biological activation of hypoxia responsive elements in the transcriptional regulation of the CYP2B6 in meduloblastoma cells.** *Yazareth Julián Cruz Villegas***,** Jonathan Alcántar Fernández, Jesús Valencia Cervantes, Juan Miranda Ríos, Rosa Angélica Castillo Rodríguez, Víctor Manuel Dávila Borja. Laboratorio de Oncología, Instituto Nacional de Pediatría |
| **GR-30** | **Identification of genes involved in the pathogenicity of the plant pathogenic fungus *Macrophomina phaseolina* in different hosts.** *Juan Angel Cuevas Moreno***,** Lucila Méndez Morán, Juan Manuel González Prieto. Centro de Biotecnología Genómica. Instituto Politécnico Nacional |

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| **GR-31** | **Evolutionaryhistory of thehistone acetyl transferase Gcn5.** *Juan Angel Cuevas Moreno***,** Jazmín Eliana Murcia Garzón, Juan Manuel González Prieto. Centro de Biotecnología Genómica. Instituto Politécnico Nacional |
| **GR-32** | **Role of translation initiation factors eIF4E and eIFiso4E during cold stress response in *Arabidopsis thaliana*.** Kenia Salazar Díaz, Tzvetanka Dimitrova Dinkova. Facultad de Química, UNAM |
| **GR-33** | **Functional analysis of miR-196b and its role in breast cancer radioresistance.** *Raquel Echavarría Zepeda***,** Elena Arechaga Ocampo, Abraham Pedroza Torres, Carlos Pérez Plascencia, Laurence A. Marchat, César López Camarillo. Posgrado en CienciasGenomicas. UACM |
| **GR-34** | **Involvement of Pygopus2 on the regulation of migration and invasion capabilities of cervical cancer cell lines.** *Luis Manuel Espinosa Sánchez***,** García Castillo Verónica, López Urrutia Eduardo and Pérez Plasencia Carlos. FES Iztacala, UNAM |
| **GR-35** | **Design and construction of an inducible system locus-directed to produce DSB in *Giardia duodenalis*.** *Sara Espinoza Corona***,** Rosa María Bermúdez Cruz. CINVESTAV Zacatenco |
| **GR-36** | **Twin a Component of the CCR4-NOT Complex, is involved in Thermal Nociception in *Drosophila*.** *Iván Fernandez Cruz,* Enrique Reynaud Garza**.** Instituto de Biotecnología, UNAM |
| **GR-37** | **Transcriptional profiling of the female reproductive tract differentiation process in mice.** *Fernando Fernández Ramírez***,** Alejandro Marmolejo Valencia, Mónica Ruíz Rosario, Ruth Ruiz Esparza Garrido, Ana Claudia Velázquez Wong, Omar Sepúlveda Robles, Diego Julio Arenas Aranda, Susana Kofman, Horacio Merchant Larios. Unidad de Genética. Hospital General de México "Dr. Eduardo Liceaga" |
| **GR-38** | **Cellular localization of 5S rRNA genes and transcripts in the parasite *Leishmania major*.** *Luis Enrique Florencio Martínez***,** Rodrigo Moreno Campos, Rebeca Manning Cela, Santiago Martínez Calvillo. FES Iztacala, UNAM |
| **GR-39** | **Molecular characterization of the effect of ovarian-specific transcription factors on the activity of *Fbxw15* promoter.** *Laura Ivette Flores Alonso,* Luis Alberto García Ávila, Norma Angélica Oviedo de Anda, Ricardo Félix Grijalva, Elsa de la Chesnaye Caraveo. Hospital de Cardiología, CMN SXXI, IMSS. CINVESTAV Zacatenco |
| **GR-40** | **Deciphering the Role of H3K9 Methylation in Plant Embryo Development.** *Marcelina García Aguilar***,** Daniel Lepe Soltero, Dao quan Xiang, Raju Datla C., Stewart Gillmor. LANGEBIO.CINVESTAV Irapuato |
| **GR-41** | **Construction of a CRISPR/Cas9 system for gene editing in *Giardia duodenalis*.** *Eduardo García Huerta***,** Rosa María Bermúdez Cruz. Genética y Biología Molecular, CINVESTAV Zacatenco |
| **GR-42** | **The role of MicroRNAs in *Marchantia polymorpha* during water deficit**. *Alma Jenny García Mejía***,** Damaris Godínez Vidal, Mario Arteaga Vázquez, Alejandra Covarrubias Robles, José Luis Reyes Taboada. Instituto de Biotecnología, UNAM |
| **GR-43** | **Identification of production of alkyl-quinolones molecules by *Pseudomonas aeruginosa* strains with an atypical quorum sensing system.** *Selene García Reyes,* Abigail González Valdez, Estefanía Morales Ruiz, Miguel Cocotl Yáñez, Samuel Gómez Pérez, Gloria Soberón Chávez. IIB. UNAM |
| **GR-44** | **MicroRNAs profiling identify miR-143 as a novel predictor of pathological complete response to chemotherapy in triple negative breast cancer**. *Raúl García Vázquez***,** Erika Ruiz García, Sergio Rodríguez Cuevas, Laurence A. Marchat, Ángeles Carlos Reyes, César López Camarillo. Posgrado Ciencias Genómicas, Universidad Autónoma de la Ciudad de México |
| **GR-45** | **A comparative analysis of perinatal disorders and DNA methylome profiling at early childhood: implementation of a pilot study.** *Joaquín Gloria Piña***,** Felipe Vadillo Ortega, Erika Chavira Suárez. FES Cuautitlán, UNAM |
| **GR-46** | ***Saccharomyces cerevisiae* longevity by depletion of Leu3 is Mediated by Gcn4.** *James González Flores***,** Stefany Argueta, Geovani López, and Alicia González. Instituto de Fisiología Celular, UNAM |
| **GR-47** | **Functional analysis of the EhTRF-like III protein of *Entamoeba histolytica***. *Díaz Medina V*, Álvarez Hernández V, Rendón Gandarilla FJ, Cárdenas Hernández H, Castañeda Ortiz EJ, Valdés Flores J, Orozco E, García Rivera G, Betanzos Fernández A, Cárdenas E, Azuara Liceaga E. Posgrado en Ciencias Genómicas, Universidad Autónoma de la Ciudad de México |

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| **GR-48** | **The miR-143/145 cluster regulates the actomyosin cytoskeleton dynamics in prostate cancer cells.** *Alejandro Gonzalez Torres***,** Eric Sulpice, Stephanie Combe, Luis Marat Alvarez Salas, Xavier Gidrol. CINVESTAV Zacatenco |
| **GR-49** | **Identification of the AtPAO2 uORF peptide in Arabidopsis seedlings.** *María de la Luz Guerrero González***,** María Azucena Ortega Amaro, Diana Sanchez Rangel, Itzell E. Hernández Sánchez, Pablo Delgado Sánchez, Margarita Rodríguez Kessler and Juan Francisco Jiménez Bremont. Universidad Autónoma de San Luis Potosí |
| **GR-50** | **Inhibition of primary myoblast differentiation by *Trichinella spiralis* muscle larvae excretory-secretory products.** *Lizbeth Hernández Ancheyta***,** María del Rosario Salinas Tobón, Javier Hernández Sánchez. Genética y Biología Molecular, CINVESTAV Zacatenco |
| **GR-51** | **Transcriptional regulation under normal and modified wort fermentation of two yeast strains used in beer industry.** *Daniel Hernández Bañuelos***,** Cintia Gómez Muñoz, Esmeralda Pérez Ortega, Luis Cástulo Damas Buenrostro, Lina Raquel Riego Ruiz. IPICyT |
| **GR-52** | **Functional characterization of *Candida tropicalis MNN4, OCH1 and PMR1*.** *Marco Josué Hernández Chávez***,** Héctor Manuel Mora Montes, Bernardo Franco Bárcenas. Departamento de Biología, DCNyE. Universidad de Guanajuato |
| **GR-53** | **Functional analysis of the silencing protein Abf1 in the fungal pathogen *Candida glabrata.*** *Grecia Hernández Hernández***,** Leonardo Castañedo Ibarra, Alejandro De Las Peñas Nava and Irene Castaño Navarro. Instituto Potosino de Investigación Científica y Tecnológica, A.C. |
| **GR-54** | **The role of SERCA pumps in the resveratrol-mediated antitumoral effect in breast cancer cells**. *Eduardo de Jesús Izquierdo Torres***,** Ángel Zarain Herzberg, Gabriela Rodríguez Rodríguez. Laboratory of Molecular Biology, Department of Biochemistry, Faculty of Medicine, UNAM |
| **GR-55** | **Transcriptional factor TFIIB1 is involved in the response to osmotic stress in *Arabidopsis thaliana.*** *Dulce Jared Jaime Gallardo,* José Antonio Miranda Ríos, Mario Ramírez Yáñez, Nancy Sofía Hernández Bueno, José Augusto Ramírez Trujillo, Ramón Suárez Rodríguez. Centro de Investigación en Biotecnología. Universidad Autónoma del Estado de Morelos |
| **GR-56** | **Role of Gln3, Gat1 and Ure2 in the regulation of nitrogen metabolism in *Lachancea kluyveri.*** *José Ángel Jiménez Benítez,* Francisco Pérez de los Santos, Lina Raquel Riego Ruiz. Instituto Potosino de Investigación Cientifica y Tecnológica |
| **GR-57** | **Analysis of small RNA biogenesis machinery expression in maize somatic embryogenesis induction.** *Vasti Thamara Juárez González,* Tzvetanka Dimitrova Dinkova. Departamento de Bioquímica, Facultad de Química. UNAM |
| **GR-58** | **Molecular characterization of the *SIR* complex in *Candida glabrata* clinical isolates.** *Osney Leiva Pelaez,* Alejandro de las Peñas Nava, Irene Castaño Navarro. Instituto Potosino de Investigacion Cientifica y Tecnologica |
| **GR-59** | **Basidiocarp development in *Ustilago maydis*: A transcriptional approach.** *Claudia Geraldine León Ramírez***,** José Luis Cabrera Ponce, Domingo Martínez Soto, José Alejandro Sánchez Arreguín, and José Ruiz Herrera. Departamento de Ingeniería Genética, CINVESTAV Irapuato |
| **GR-60** | **Patterns of gene activation in hybrid embryos of *Arabidopsis thaliana*.** *Daniel Lepe Soltero,* Cei Abreu Goodger & Stewart Gillmor. Unidad de Genómica Avanzada. LANGEBIO. CINVESTAV Irapuato |
| **GR-61** | **Does the accumulation of trehalose in *Ustilago Maydis* change the virulence and the response to stress?.** *Alonso López Cabrera,* Edgardo Ulises Esquivel Naranjo, Fidel Landeros Jaime, José Antonio Cervantes Chávez. Universidad Autónoma de Querétaro |
| **GR-62** | **Expression profiles of tasiR-ARFs pathway-related genes during maize somatic embryogenesis.** *Brenda Anabel López Ruiz,* Tzvetanka Dimitrova Dinkova. Departamento de Bioquímica. Facultad de Química, UNAM |
| **GR-63** | **Locus-specific *Rxrα* promoter methylation analysis and its expression in the offspring's umbilical cord from high-fat diet-induced obese rat.** *Itzel Ivonn López Tenorio,* Elena Zambrano González, Luis Reyes Castro, Aarón Domínguez López, Felipe Vadillo Ortega, Erika Chavira Suárez. Escuela Superior de Medicina, IPN |

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| **GR-64** | **Understanding *Candida glabrata* resistance to oxidative stress through CTA1 gene regulation.** *Gabriel Guillermo Luna Arvizu,* Israel Cañas Villamar, Irene Castaño Navarro, Alejandro de las Peñas Nava. IPICYT |
| **GR-65** | **Stem loop: Molecular technique used for isolation and identification of small RNAs from *Giardia lamblia*.** *Jaime Marcial Quino,* Saúl Gómez Manzo, Edgar Sierra Palacios, Erick Alcaraz Carmona, Yadira Yazmín Cortés Morales, Estefania Millán Ramírez de Arellano, Laura Eloisa Morales Luna, Karina Pérez Nuñez, Edson Jiovany Ramírez Nava, María Fernanda Trejo Martínez, Horacio Reyes Vivas. Instituto Nacional de Pediatría |
| **GR-66** | **Characterization of KH-QUA2 domain of the U2 snRNP auxiliary factor 84 kDa (U2AF84) of *Entamoeba histolytica*.** *Ricardo Martínez Baltazar,* Lorena Palacio Molina, Carlos Ortuño Pineda, Nicolás Villegas Sepúlveda, María Saraí Mendoza Figueroa, Emmanuel Reyes Castro, José Manuel Galindo Rosales, Jesús Valdés Flores. CINVESTAV, IPN |
| **GR-67** | **Sweet taste perception genes: TAS1R2 and GLUT2 polymorphisms, carbohydrate intake and nutritional state in Mexican adults.** *José Darío Martínez Ezquerro,* Isabel Miriam Adriano Quintana, Deyanira Escalante Bautista, Beatriz Rosales Rodríguez, Haydeé Rosas Vargas. Centro Medico Nacional "Siglo XXI" IMSS |
| **GR-68** | **Analysis of phasin-like protein on Polyhydroxy butyrate production in *Azospirillum brasilense* Sp7.** *María de los Ángeles Martínez Martínez,* Itzel Anaya Benítez, Lucía Soto Urzúa, Luis Javier Martínez Morales. Universidad Autónoma de Puebla |
| **GR-69** | **Gene regulation in *Ustilago maydis* by a MAPK pathway involved in pathogenesis, mating and morphogenesis.** *Domingo Martínez Soto,* José Ruiz Herrera.CINVESTAV. Unidad Irapuato |
| **GR-70** | **Identification of low molecular weight excretory-secretory products from *Trichinella spiralis* muscle larvae potentially involved in the inhibition of myotube formation in primary myoblast cultures.** *Krystal Maya Maldonado,* María del Rosario Salinas Tobón, Javier Hernández Sánchez. CINVESTAV. Zacatenco |
| **GR-71** | **Physiological roles for MXL-3/MAX as lipogenic factor that modulate organismal fat accumulation.** *Fanny Mejía Martínez,* Berenice Franco Juárez, Elizabeth Moreno Arriola, Alain de Jesús Hernández Vázquez, Antonio Velázquez Arellano, Karla Carvajal, Daniel Ortega Cuellar. Instituto Nacional de Pediatría |
| **GR-72** | **Germ-line BRCA 1 and 2 mutations in breast and ovarian cancer patients.** *Oliver Millán Catalán,* Abraham Pedroza Torres, Eduardo López Urrutia, Carlos Pérez Plasencia. Instituto Nacional de Cancerología |
| **GR-73** | **Functional interactions of NFAT, NF-kB and Sp1 proteins regulate the expression of IL-10 in U937 monocytes.** *Lourdes Millán Pérez Peña,* Jorge A. Calzada Martínez, Sandra R. Reyes Carmona, Nora H. Rosas Murrieta, Irma P. Herrera Camacho, Eduardo M. Salinas Stefanon, Thomas Scior. Instituto de Ciencias, Universidad Autónoma de Puebla |
| **GR-74** | **Postranscriptional regulation of Cyclin D1 protein in cells transfected with E6 oncogene from HPV type 6, 16, 18, and 52.** *Vicente Morales García,* Adriana Contreras Paredes, Alma Chávez Blanco, Daniel Jímenez Martínez, Erick De la Cruz Hernández. Universidad Juárez Autónoma de Tabasco |
| **GR-75** | **Molecular classification of histonemetyl transferases.** *Jazmín Eliana Murcia Garzón,* Gustavo Hernández Guzmán, Luis José Delaye Arredondo, Juan Manuel González Prieto. Centro de Biotecnología Genómica, IPN |
| **GR-76** | **Phenotypic and transcriptomic analysis of the *atx1-1$^{setm}$* mutant affected in lateral root development.** *Selene Napsucialy Mendivil,* Svetlana Shishkova, Joseph G. Dubrovsky. Instituto de Biotecnología, UNAM |
| **GR-77** | **The *TIME FOR COFFEE* gene participates in the hydrotropic response regulation in *Arabidopsis thaliana*.** *Laura Noriega Calixto,* María Eugenia Campos Torres, Delfeena Eapen, Gladys I. Cassab López. Instituto de Biotecnología, UNAM |
| **GR-78** | **HLH-30/TFEB together $NAD^+$ relieves the blocked autophagic flux due to high glucose diet.** *Berenice Franco Juárez*, Fanny Mejía Martínez, Elizabeth Moreno Arriola, Alain de Jesús Hernández Vázquez, Antonio Velázquez Arellano, Karla Carvajal, Daniel Ortega Cuellar. Instituto Nacional de Pediatría |

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| **GR-79** | ***Phaseolus vulgaris* calreticulin: unraveling its role in nodulation.** *Yolanda Ortega Ortega,* Ricardo Omar Vazquez Consejo, Angélica Lucía Martínez Aguilar, Marco Adán Juárez Verdayes, Noreide Nava, Xóchitl Alvarado, Olivia Santana, Alfonso Leija, Carmen Quinto. Instituto de Biotecnología, UNAM |
| **GR-80** | **A novel class of non-coding RNAs in *Entamoeba histolytica*.** *Lorena Palacio,* Ricardo Martínez Baltazar, María Saraí Mendoza Figueroa, Jesús Valdés. CINVESTAV, IPN |
| **GR-81** | **Functional Analysis of Mir-122 on Radioresistance of the Breast Cancer Cells.** *Isidro Xavier Pérez Añorve,* Reynalda Roldán Pérez, Claudia Haydée González de la Rosa, Elena Aréchaga Ocampo. Universidad Autónoma Metropolitana, Unidad Cuajimalpa |
| **GR-82** | **GNAO1 and ASAH1 as probable biomarkers for pediatric ependymoma.** *Monserrat Pérez Ramírez,* Diego Alberto Enríquez Sinta, Hernández Jiménez Alejo Justino, Del Ángel Ruiz Israel, Agustín Aguilar Fernando, Siordia Reyes Alicia Georgina, García Méndez Antonio, Chico Ponce de León Fernando, Salamanca Gómez Fabio Abdel, García Hernández Normand. Centro Medico Nacional "Siglo XXI" IMSS |
| **GR-83** | **Analysis of the Genetic Diversity of *Ustilago maydis* in Mexico using Microsatellite Markers.** *Víctor Hugo Ramos García,* María Fernanda Jiménez Becerril, María Myrna Solís Oba, Sanjuana Hernández Delgado, Juan Manuel González Prieto. Centro de Biotecnología Genómica, IPN |
| **GR-84** | **Ribosomal profiles and rRNA synthesis in mutants (ΔRPP1A, ΔRPP1B, ΔRPP2A and ΔRPP2B) affecting ribosomes acidic proteins of *Saccharomyces cerevisiae*.** *Juan Ismael Rea Hernández,* Arnulfo Bautista Santos, Samuel Zinker Ruzal. Departamento de Genética y Biología Molecular, CINVESTAV, IPN |
| **GR-85** | **Role of the DisA-UvrABC interaction in protecting germinating/outgrowing *Bacillus subtilis* spores from oxidative promoted-DNA damage.** *Ana Gabriela Regalado García,* Luz Idalia Valenzuela García, Mario Pedraza Reyes. Universidad de Guanajuato |
| **GR-86** | ***Candida glabrata* encodes a longer variant of the mating type (*MAT*) alpha2 gene in the mating type-like *MTL3* locus.** *Karina Asyade Robledo Márquez,* Gutiérrez Escobedo Guadalupe, Yañez Carrillo Patricia, Briones Martín del Campo Marcela, Orta Zavalza Emmanuel, De Las Peñas Nava Alejandro, Castaño Navarro Irene. Instituto Potosino de InvestigaciónCientífica y Tecnológica A.C. |
| **GR-87** | **Mutation in *TOR* affect leaf margin and pod size in *Lotus japonicus* by altering *PIN1*, *KNOTTED1*, *KNOX1* and PHAN expression.** *Fatima Arizbeth Plascencia,* Kalpana Nanjareddy, Miguel Lara, Lourdes Blanco, Manoj Kumar Arthikala. ENES. León, UNAM |
| **GR-88** | **Influence of hydrogenperoxide ($H_2O_2$) in the regulation of the Phtcluster of *Pseudomonas syringae*pv. phaseolicola NPS3121.** *Jennifer Alexis Rojas Morales,* Alejandro Hernánde zMorales, Jesús Bernardino Velázquez Fernández, Verónica Alejandra Mondragón Jaimes, Jackeline Lizzeta Arvizu Gómez. Universidad Autónoma de Nayarit |
| **GR-89** | **Role of Bdp1 in RNA Polymerase III transcription in *Leishmania major*.** *Fiordaliso C. Román Carraro,* Luis E Florencio Martínez, Rebeca Manning Cela, Santiago Martínez Calvillo. FES Iztacala, UNAM |
| **GR-90** | **Role of *GRHd3* Genetic Variant in Reducing Weight in Morbidly Obesity Patients.** *Andy Michel Romero Nieves,* Osvaldo D Castelán Martínez, Etual Espinosa, Martha A Sánchez Rodríguez, Claudia Ramírez Rentería, Mario Molina Ayala, Moisés Mercado. FES Zaragoza, UNAM |
| **GR-91** | **Analysis of functional status of c-Met receptor and its relationship with stemness and invasive capacity of a small subpopulation derived from cell lines of gastric cancer.** *Claudia Ivette Rugerio Martínez,* Leny Palma López, Damaris Romero, Alejandro García Carrancá, Luis A. Herrera Montalvo, José Efrain Garrido Guerrero, Elizabeth Ortiz Sánchez, Instituto Nacional de Cancerología |
| **GR-92** | **MicroRNAs-mediated regulation of the tumor suppressor Merlin in response to inflammatory signals.** *Nilda del Carmen Sánchez Castellanos,* Karla Meza Sosa, Leonor Pérez Martínez, Gustavo Pedraza Alva. Instituto de Biotecnología, UNAM |
| **GR-93** | **Role of translation initiation factors eIF4E and eIFiso4E during cold stress response in *Arabidopsis thaliana*.** *Kenia Salazar Diaz,*Tzvetanka D Dinkova.Facultad de Química, UNAM |
| **GR-94** | **Transcriptional changes occurring in nitrogen fixation by an endosymbiotic bacteria in the basidiomycota fungus *Ustilago maydis*.** *José Alejandro Sánchez Arreguín,* Claudia Geraldine León Ramírez, Miguel Ángel Hernández Oñate, José Ruiz Herrera. CINVESTAV. Unidad Irapuato |

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| **GR-95** | ***Ustilago Maydis* gene *NRG1* identification and deletion.** *José Alejandro Sánchez Arreguín,* José Ruiz Herrera, Elva Teresa Aréchiga Carvajal. Universidad Autónoma de Nuevo León |
| **GR-96** | **Deleting a gene in a hard to transform yeast. The story of *Debaryomyces hansenii.*** *Norma Silvia Sánchez Sánchez,* Martha Calahorra Fuertes, Laura Kawasaki Watanabe, Roberto Coria Ortega, Nicolas Papon, Tatiana Defosse, Antonio Peña. Instituto de Fisiología Celular, UNAM |
| **GR-97** | ***Ustilago maydis* metacaspase is involved in response to stress.** *Cinthia Valentina Soberanes Gutiérrez,* Evelia Judith Figueroa Reséndiz, Edgardo Ulises Esquivel Naranjo, Fidel Landeros Jaime, José Antonio Cervantes Chávez, José Ruiz Herrera. CINVESTAV. Unidad Irapuato |
| **GR-98** | **Feronia gene has a significant role in the *Phaseolus vulgaris*- rhizobia symbiotic interaction.** *Jorge Solís Miranda,* Marco Juárez, Carmen Quinto. Instituto de Biotecnología, UNAM |
| **GR-99** | **Regulation of expression of the *nrdEF* operon encoding the class 1b ribonucleotide reductase in *Bacillus subtilis*.** *Valeria Patricia Suárez Castro,* Karla Viridiana Castro Cerritos, Mario Pedraza Reyes. Departamento de Biología, Universidad de Guanajuato |
| **GR-100** | **CRISPR/Cas9 mutagenesis of *Sporothrix schenckii RmlD*.** *Alma Karina Tamez Castrellón,* Luz Adriana López Ramírez, Héctor Manuel Mora Montes. Departamento de Biología, Universidad de Guanajuato |
| **GR-101** | **Study of genetic variability in virulence factors among strains of *Avibacterium paragallinarum*.** *Horta Valerdi Guillermo Manuel,* Negrete Abascal Erasmo, Sánchez Alonso Ma. Patricia, Vázquez Cruz Candelario. Universidad Autónoma de Puebla |
| **GR-102** | **Sum1 as a virulence factor in *Candida glabrata*.** *Norma C Vázquez Franco,* Emmanuel Orta Zavalza, Irene Castaño, Alejandro De Las Peñas. Instituto Potosino de Investigación Científica y Tecnológica, A.C. |
| **GR-103** | **Construction of vectors for *MTL* gene expression in *Candida glabrata*.** *Yamile Vidal Aguiar,* Gutiérrez Escobedo Ma. Guadalupe, De Las Peñas Alejandro, Castaño Navarro Irene. Instituto Potosino de Investigación Científica y Tecnológica, A.C. |
| **GR-104** | **Role of the Oxidative Stress in the expression of the Pht cluster genes involved in the phaseolotoxin synthesis in P. *Syringae* pv. Phaseolicola NPS3121.** *Marisol Reynoso López*, Alejandro Hernández Morales, Abril Bernadette Martínez Rizo, José Navarro Partida, Jackeline Lizzeta Arvizu Gómez. Universidad Autónoma de Nayarit |
| **GR-105** | **mRNA expression of the glutathione related genes in cultured HeLa cells treated with valproic acid.** *Benjamín González López*, Reyna Elizabeth Barbosa Cabrera, Ismael Vásquez Moctezuma. ESM Instituto Politécnico Nacional |

## MICROBIOLOGY AND VIRUSES

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| **MV-1** | **Effect of the E6 and E7 oncoproteins of the HPV18 in the genes expression associated whit the glycosylation.** *Miguel Ángel Aco Tlachi*, Ricardo Carreño López, Adriana Aguilar Lemarroy, Luis Felipe Jave Suárez, Gerardo Santos Lopez, Paola Maycotte González, Flores Alonso Juan Carlos, Veronica Vallejo Ruiz. Centro de Investigaciones en Ciencias Microbiológicas, Universidad Autónoma de Puebla |
| **MV-2** | **Characterization of the cellular receptors diversity for astrovirus.** *Nayeli Aguilar Hernández,* Carlos Federico Arias López, Oscar Trejo Cerro. Instituto de Biotecnología, UNAM |
| **MV-3** | **Dynamics of antagonistic interactions in an invasive network of bacteria.** *Bernardo Aguilar Salinas,* Gabriela Olmedo Álvarez. CINVESTAV Irapuato |
| **MV-4** | **The c-di-GMP protein MucR is necessary for cyst formation but not for alginate synthesis in *Azotobacter vinelandii*.** *Iliana Chantal Martínez Ortiz*, Josefina Guzmán Aparicio, Cinthia Ernestina Núñez López. Centro de Ciencias Genómicas, UNAM |
| **MV-5** | **Microbial consortia with different biochemical capabilities for hydrocarbons degradation.** *Cesar Alvarez Mejia,* Felipe Cerratos Hernández, Martha Isela Gutiérrez Amézquita, Varinia López Ramírez. Coordinación de Ingeniería Ambiental, Instituto Tecnológico Superior de Abasolo |

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| **MV-6** | **Isolation and identification of triterpenoid acids with antitumoral effect in a submerged culture of *Humphreyacoffeata***. *Viridiana Asprón Moncada***,** Monserrat García García Norma A. Valdez Cruz Mauricio A. Trujillo Roldán. Instituto de Investigaciones Biomédicas, UNAM |
| **MV-7** | **Characterization of putative antiterminator gene e46 of bacteriophage mEp021.** *Elissa Paulina BallinasTurrén***,** Eva Martínez Peñafiel, Luis Kameyama. Departamento de Genética y Biología molecular. CINVESTAV Zacatenco |
| **MV-8** | **HilD and PhoP regulate the expression of a novel gene required for *Salmonella enterica*serovar Typhimurium invasion of host cells**. *María Magdalena Banda Hernández***,** Rubiceli Manzo, Jay C. D. Hinton, José L. Puente, Fernando C. Soncini, Francisco García del Portillo, Víctor H. Bustamante. Departamento de Microbiología Molecular, Instituto de Biotecnología, UNAM |
| **MV-9** | **Pathogenicity genes and phylogenetic group in uropathogenic *Escherichia coli* strains.** *Juan Carlos Bravata Alcántara,* Juan José Méndez Velázquez, Iliana Alejandra Cortés Ortiz, Concepción Cu Quijano, Mónica Sierra Martínez. Unidad de Genética y Cáncer. Laboratorio Central, Hospital Juárez de México |
| **MV-10** | **Microbial volatile organic compounds (mVOCs) that promote growth in *Arabidopsis thaliana.*** *David Alfonso Camarena Pozos*, Mercedes Guadalupe López Pérez, José López Bucio, Juan José Peña Cabriales and Laila Pamela Partida Martínez. CINVESTAV Unidad Irapuato |
| **MV-11** | **Isolation, identification and characterization of nitrogen-fixing bacteria from the rhizosphere of plants inhabiting the mine "El Bote" from Zacatecas, Zac. Mexico.** *Alexandro Castanon Quevedo*, Juan Armando Flores de la Torre, Rosa Maria Ramirez Santoyo, Jesus Guzman Moreno, Luz Elena Vidales Rodriguez. Universidad Autónoma de Zacatecas "Francisco García Salinas" |
| **MV-12** | **Phenolic compounds and antimicrobial activity of aqueous extracts of *Lippia alba* and *Lippia dulcis* of Cunduacán, Tabasco.** *Tania Paulina Carrasco de la Cruz***,** Blanca Flor Ocampo Medina, Daniel Alejandro Vázquez Cahuich, José Rodolfo Velázquez Martínez, Judith Espinoza Martínez, Dora Centurión Hidalgo.Ciencias Básicas, Universidad Juárez Autónoma de Tabasco |
| **MV-13** | **Detection of Influenza A virus canine in the metropolitan area of Monterrey, Nuevo Leon.** *Juan Francisco Contreras Cordero***,** Claudia Bernardette Plata Hipólito, Sibilina Cedillo Rosales, Carlos Eduardo Hernández Luna, Cristina Rodríguez Padilla, Reyes Tamez Guerra. Facultad de Ciencias Biológicas. Universidad Autónoma de Nuevo Léon |
| **MV-14** | **Infection with influenza A virus H1N1 (2009) of endothelial cells HMEC-1 and its effect on Protease Activated Receptors (PAR-1).** *Guillermo Cordero García,* Fernando Hernández Sánchez, Pedro Vargas Leonor, Luis Ángel Pérez Moreno, José Luis Eduardo Flores Sáenz, Carlos Cabello Gutiérrez, UAMI. Instituto Nacional de Enfermedades Respiratorias Ismael Cosio Villegas |
| **MV-15** | **Isolation of electrochemically active bacteria from a microbial fuel cell.** *Alan Jacob Cornejo Martell*, María Yolanda Reyes Vidal, Francisco Javier Bácame Valenzuela, Bibiana Cercado Quezada, Julián Peña Castro. Centro de Investigación y Desarrollo Tecnológico en Electroquímica SC. |
| **MV-16** | **Use of the MALDI-TOFMS Biotyper system for rapid identification of microbial strains isolated from pesticide-contaminated soil in Salamanca, Gto.** *Alma Rosa Corrales Escobosa,* Bianey Garcia Lara, Ma. Fernanda Morales Gutierrez, Armando Alcazar Magaña Kazimierz Wrobel, Katarzyna Wrobel. Departamento de Química. DCNE. Universidad de Guanajuato |
| **MV-17** | **Functional characterization of GrlR, a LEE-encoded negative regulator of enteropathogenic *Escherichia coli.*** Emma Aurora Cruz Gómez**,** José Luis Puente. Departamento de Microbiología Molecular. Instituto de Biotecnología, UNAM |
| **MV-18** | **Incidence of *Demódex folliculorum* infestation in students from the Universidad Juárez Autónoma de Tabasco, campus Chontalpa.** *María Guadalupe de los Santos López***,** Keren López Pérez, Olga Selene Vidal Lucas, Miguel Ángel Gómez López, Daniel Alejandro Vázquez Cahuich, Patricia Mendoza Lorenzo. División Académica de Ciencias Básicas.Universidad Juárez Autónoma de Tabasco |
| **MV-19** | **Microbiological analysis of water intended for human consumption in the municipality of Centro Tabasco**. Yisel Diaz Caliz, Solaina Del Lucero Jiménez López, Mayra Alondra Jiménez López, Laura Fabiola Estrada Andrade. Universidad Juárez Autónoma de Tabasco |

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| **MV-20** | **Absence of alcohol dehydrogenase (ADH1) activity in *Mucorcircinelloides* increases the mouse tissue invasivity of the fungus.** *Sharel Pamela Díaz Pérez***,** Marco I. Valle Maldonado, Pamela Romo Rodríguez, Adolfo López Torres, Irvin E. Jacome Galarza, Jesús Campos García, Rafael Ortíz Alvarado, J. Félix Gutiérrez Corona, Víctor Meza Carmen. Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolas de Hidalgo |
| **MV-21** | **Toxin genes PirA and PirB type in *Vibrio parahaemolyticus* strains isolated from shrimp.** *Ma. de Jesús Durán Avelar***,** Norberto Vibanco Pérez, Ana Lourdes González Mercado, José Francisco Zambrano Zaragoza, Víctor Alfonso Sánchez Chávez, Zulia Fernandina Nieves López. Autonomous University of Nayarit. Academic Unit of Chemical Biological and Pharmaceutical Sciences |
| **MV-22** | **The HPV16 E6 oncoprotein variants affects the expression of cadherins in cell C33-A cells.** *Estela Ramírez Valeria***,** Arcos Almazán José Mauricio, Sollano Mendieta Citlali E, Hernández Sotelo Daniel, Illades Aguiar Berenice, Del Moral Hernández Oscar. Laboratorio de Virología, Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Guerrero |
| **MV-23** | **Dissecting microbiome functions in cacti: lessons from seed-transmitted endophytes.** *Víctor M. Flores Núñez*, Citlali Fonseca García & Laila P. Partida Martínez. CINVESTAV. Unidad Irapuato |
| **MV-24** | **Isolation and identification of seed-associated bacteria from Guamuchil (*Pithecellobium dulce* (Roxb) Benth.).** *Citlalli Gallegos Bolaños,* Francisco Luna Martínez, Fermín P. Pacheco Moisés, Celso Cortés Romero. CUCEI, Universidad de Guadalajara |
| **MV-25** | **Antibody generation against the recombinant protein Gp70 from *Sporothrix schenckii* sensus tricto and *Sporothrix brasiliensis*.** *Laura Cristina García Carnero***,** José A. Martínez Álvarez, Nahum V. Hernández, Héctor M. Mora Montes. División de Ciencias Naturales y Exactas. Universidad de Guanajuato |
| **MV-26** | **Phenotypic diversity of *B. coahuilensis*and increase of carbon utilization in experimental evolution.** *Zulema Gómez Lunar***,** Gabriela Olmedo Álvarez. Departamento de Ingeniería Genética. CINVESTAV Irapuato |
| **MV-27** | **Recruitment of celular factor RTN3 in the viral replication complex on HMEC-1 cells infected with Dengue virus.** *Luis Didier González García***,** Saucedo Hernández José Luis, García Cordero Julio, Cedillo Barrón Leticia, León Juárez Moises. Instituto Nacional de Perinatologia Isidro Espinosa de los Reyes |
| **MV-28** | **Identification of *Rhizobium tropici* CIAT899 genes involved in Benzo[a]pyrene resistance or degradation.** *Yessica González Paredes***,** Blanca Jazmín Reyes Hernandez, Ernesto Ormeño Orrillo, Esperanza Martínez Romero. Centro de Ciencias Genómicas, UNAM |
| **MV-29** | **Effect of psidium guajava on periodontopathogenic bacteria.** *Alexandra González Ulloa,* Rubén Octavio Méndez Márquez, Blanca Patricia Lazalde Ramos. Unidad Académica de Ciencias Químicas. Universidad Autónoma de Zacatecas "Francisco García Salinas" |
| **MV-30** | **Expression of IRE1α as a prognostic marker of cervical cancer: an immunohisto chemical analysis.** *Rusland Enrique Torres Orozco*, Rafael Gutiérrez Campos. Laboratory of Virology and Genetic Engineering. Autonomous University of Aguascalientes |
| **MV-31** | **Isolation and analysis of membrane microdomains in *Escherichia coli*.** *José Enrique Guzmán Flores***,** Adrián Fernando Alvarez, Marina Gavilanes Ruiz, Sebastian Poggio Ghilarducci, Dimitris Georgellis. Instituto de Fisiología Celular, UNAM |
| **MV-32** | **Production and partial characterization of bacteriocin-like substances from different strains of the *Streptomyces* genus.** *Oscar Felipe Hernández Saldaña,* José Eleazar Barboza Corona, Luz Edith Casados Vázquez. Campus Irapuato Salamanca. Universidad de Guanajuato |
| **MV-33** | **Identification and Molecular Detection of *Staphylococcus spp*., from Uterine Cervix Scrapes.** *Reynaldo Hernández Santiago,* KeikoTaniguchi Ponciano, Daniel Marrero Rodríguez, Pablo Romero Morelos, Mariana Valdespino Zavala, Alejandra Rodríguez Castellanos, Ismael Rodríguez Idelfonso, Berenice Mulato Briones, Rosa M Ribas Aparicio, Patricia Cauich Sánchez, Juan López Esparza, María del Socorro Méndez Tovar, Annabelle Cerón Nava, Norma E Herrera González, Ricardo López Romero, Mauricio Salcedo Vargas.UMAE Hospital de Oncología CMN SXXI-IMSS. IPN. |

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| **MV-34** | **Phenotypic plasticity of *Bacillus* isolates from Coahuila, Mexico.** *Enrique Hurtado Bautista***,** Diana Fabiola Díaz Jiménez, Diana Guadalupe Tapia García, Gabriela Olmedo Álvarez. CINVESTAV Unidad Irapuato |
| **MV-35** | **Regulatory mechanisms controlling the expression of the *ecp* fimbrial operon in *Citrobacter rodentium*.** *María Inés Isidro Coxca,* Verónica I. Martínez Santos, Andrés Escalera, Abraham Medrano, Gustavo Caballero, Alejandra Vázquez, José Luis Puente. Instituto de Biotecnología, UNAM |
| **MV-36** | **Phenotypic differentiation of aquatic *Bacillus* species**. *Africa Islas Robles,* Zulema Gómez Lunar, María Dolores Rodríguez Torres, Vianney Rodríguez Razo, Diana Guadalupe Tapia García and Gabriela Olmedo Álvarez. CINVESTAV Irapuato |
| **MV-37** | **Antiviral and immunomodulatory effect of six polyphenolic compounds in cells infected with dengue virus serotype 2.** *María Carolina Jasso Miranda,* Irma Herrera Camacho, Lilian Karem Flores Mendoza, Irma Fabiola Domínguez Avilés, Nora Hilda Rosas Murrieta, Dino Gnecco Medina, Gerardo Santos López, Julio Roberto Reyes Leyva.Virology laboratory, CIBIOR-IMSS. Benemérita Universidad Autónoma de Puebla |
| **MV-38** | **Detection of *Pseudomonas aeruginosa* in spinal liquid fo rLoop-mediated Isothermal Amplification (LAMP).** *Maria del Sol Jiménez López,* Daniel Alejandro Morales Vázquez, Ana Gabriel Estrada Martinez, Juan Joel Mosqueda Gualito, Bertha Carvajal Gamez. Faculty of Natural Sciences, Autonomous University of Queretaro |
| **MV-39** | **Expression of the serine protease MarP from *Mycobacterium tuberculosis* in the periplasm of *Escherichia coli.*** *Jorge Luis Jiménez Niebla,* Alexis Zarahy Minchaca Acosta, Patricia Lilián Alejandra Muñoz Muñoz, Rosa Elena Mares Alejandre, Samuel Guillermo Meléndez López, Marco Antonio Ramos Ibarra. Facultad de Ciencias Químicas e Ingeniería, Universidad Autónoma de Baja California |
| **MV-40** | **The impact of adenoviral protein E1B-55 kDa phosphorylation on the DNA Viral replication.** *Raúl Eduardo López Antonio,* Ramón A. González García Conde. Instituto de Biotecnología. Centro de Investigación en Dinámica Celular, UAEM |
| **MV-41** | **Study on the Antifungal Activity of Silver Nanoparticles.** *Jessica López Hernández,* Ana Gabriela Estrada Almeida, Patricia Mendoza Lorenzo, Pascual Pedraza Montero, Juan Carlos Arévalo Pérez, Irma Sánchez Lombardo. Univerisidad Juárez Autónoma de Tabasco |
| **MV-42** | **Molecular characterization of *Gallibacterium anatis* -like strains harbored in hens.** *Ana Jacqueline López Ochoa***,** Anallely Cervantes Mendoza, Erasmo Negrete Abascal, Sergio Vaca Pacheco, Víctor Pérez Márquez, Armando Tapia Hernández, Teresita Jiménez Salgado, María Patricia Georgina Sánchez Alonso & Candelario Vázquez Cruz. Universidad Autónoma de Puebla |
| **MV-43** | **Photobionts diversity is related with lichens ecological distribution in Guanajuato.** *Varinia López Ramírez*, Clementina Franco Palatto, César Álvarez Mejía. Coordinación de Ingeniería Bioquímica, Instituto Tecnológico Superior de Irapuato |
| **MV-44** | **Novel fusion proteins generated with fimbrial adhesins of uropathogenic *Escherichia coli.*** *Victor Manuel Luna Pineda,* Juan Pablo Reyes Grajeda, Ariadnna Cruz Córdova, Zeus Saldaña Ahuactzi, Vicenta Cázares Domínguez y Juan Xicohtencat Cortes. Laboratorio de Investigación en Bacteriología Intestinal, Hospital Infantil de México Federico Gómez |
| **MV-45** | **Function analysis of *RSP_1318* a component of the Fla2 flagella of *Rhodobacter sphaeroides***. *Arely Ivonne Marcos Vilchis,* Teresa Ballado, Javier de la Mora, Laura Camarena, Georges Dreyfus. Instituto de Fisiología Celular, UNAM |
| **MV-46** | **Study of essential genes in *Escherichia coli.*** *Enrique Martínez Carranza,* Hugo Rafael Barajas de la Torre, Luis David Alcaraz Peraza, Luis Servín González, Gloria Soberón Chávez. Departamento de Biología Molecular y Biotecnología, Instituto de Investigaciones Biomédicas, UNAM |
| **MV-47** | **Involvement of cyclodipeptides in the bacterial population control of the Churince system of Cuatro Ciénegas basin.** *Enrique Martínez Carranza,* Gabriel Y. Ponce Soto, Valeria Souza Saldívar, Alma Laura Díaz Pérez and Jesús Campos García. Universidad Michoacana de San Nicolas de Hidalgo |
| **MV-48** | **Role of the nucleoid-associated proteins H-NS and StpA in the regulation of *Citrobacter rodentium* virulence.** *Haydee Martínez Plascencia,* Gustavo G. Caballero Flores, José Luis Puente. Instituto de Biotecnología, UNAM |

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| **MV-49** | **Expression of the sRNA´s CrcZ and CrcY in Different Carbon Catabolic Repression Conditions in *Azotobacter vinelandii.*** *Marcela Martínez Valenzuela,* Josefina Guzmán Aparicio, Cinthia Ernestina Núñez López. Instituto de Investigación en Ciencias Básicas y Aplicadas, Universidad Autónoma del Estado de Morelos |
| **MV-50** | **Study and identification of cold adaptation proteins in the Antarctic yeast *Rhodotorula mucilaginosa.*** *Ana Paulina Mendoza Von der Borch,* Jorge Brito Sánchez, Claudia Segal Kischinevzky, Víctor Valdés López, Viviana Escobar Sánchez, Alfonso Vilchis Peluyera, Marcelo Baeza, Luisa Alba Lois. Facultad de Ciencias, UNAM |
| **MV-51** | **Clonning and expression of Dengue virus protein NS4A.** *Daniel Montes Herrera,* Saucedo Hernández José Luis, Ramos Rojo Raymundo, Helguera Repetto Cecilia, Garcia Cordero Julio, Cedillo Barrón Leticia, León Juárez Moises. Laboratorio de Inmunobioquímica, Instituto Nacional de Perinatologia Isidro Espinosa de los Reyes |
| **MV-52** | **Characterization of the quorum-sensing system RhlR/RhlI of a *Pseudomonas aeruginosa* dolphin isolate.** *Estefanía Morales Ruiz,* Luis Servín González, Gloria Soberón Chávez. Instituto de Investigaciones Biomédicas, UNAM |
| **MV-53** | **Fungal endophytes diversity associated to *Pytogramma sp*., thermotolerant fern from "Los Azufres", Michoacán**. *Adán Topiltzin Morales Vargas,* Cesar Álvarez Mejía, Segoviano Santoyo Fátima Guadalupe, Varinia López Ramírez. Ingeniería Agroindustrial, División de Ciencias de la Salud e Ingenierías, Universidad de Guanajuato |
| **MV-54** | **Association between *Helicobacter pylori* infection and periodontitis.** *Luis Enrique Ordoñez Mejia,* María Olivia Medel Flores, María del Consuelo Gómez García, David Guillermo Pérez Ishiwara, Nury Pérez Hernández, Virginia Sánchez Monroy. Escuela Nacional de Medicina y Homeopatía, Instituto Politécnico Nacional |
| **MV-55** | **Endophytic fungus with antibiotic activity against fungal plant-pathogens.** *Claudia Ordóñez Valencia,* Rufina Hernández Martínez. CICESE |
| **MV-56** | **Analysis of the effect of nucleolin and CTCF in adenovirus 5 replication.** *Yoatzin Peñaflor Téllez,* Ramón A. Gonzalez. Instituto de Investigación en Ciencias Básicas y Aplicadas, UAEM |
| **MV-57** | **Isolation and characterization of microorganism with agronomic importance from the rhizosphere of "maguey pulquero" (*Agave salmiana*).** María del Pilar Osorno Suárez, Teresa Romero Cortes, Martín Peralta Gil, Raúl Noguez Moreno, Gustavo Hernández Guzmán, Julio Vega Arreguín, Noé Duran Figueroa, Jaime Bravo Ramírez, *Victor Hugo Pérez España*. Escuela Superior de Apan, Universidad Autónoma del Estado de Hidalgo |
| **MV-58** | **Novel methodology to fractionate cell proteins for two-dimensional gel electrophoresis.** *Luis Angel Pérez Moreno,* Fernando Hernández Sánchez, Héctor Guillermo Cordero García, Pedro Vargas Leonor, Carlos Cabello Gutiérrez. Instituto Nacional de Enfermedades Respiratorias Ismael Cosio Villegas |
| **MV-59** | **Neutralizing activity of anti-M1 antibodies against equine influenza virus.** *Claudia Bernardette Plata Hipólito,* Sibilina Cedillo Rosales, Licet Villarreal Treviño. Cristina Rodríguez Padilla, Reyes Tamez Guerra, Juan Francisco Contreras Cordero. Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo Léon |
| **MV-60** | **Searching for the function of an early protein from bacteriophage PaMX41 infecting *Pseudomonas aeruginosa*.** *Irais Ramírez Sánchez,* Indira Cruz Plancarte, Gabriel Guarneros. CINVESTAV Zacatenco |
| **MV-61** | **The use Killer's toxin from yeast *S. cerevisiae* as an inductor of cellular death in microorganisms with biomedical importance.** *Jimena Ramírez Villarreal,* Juan Campos Guillen, Verónica Morales Tlalpan, Jorge Chávez Servín, Roberto Ferriz Martínez & Carlos Saldaña. Facultad de Ciencias Naturales. Universidad Autónoma de Querétaro |
| **MV-62** | **Analysis of the disulfide oxidoreductase activities of a DsbA-like protein from *Mycobacterium tuberculosis*.** *Marco Antonio Ramos Ibarra,* Alexis Zarahy Minchaca Acosta, Pablo Alfonso Madero Ayala, Patricia Lilián Alejandra Muñoz Muñoz, Samuel Guillermo Meléndez López, Rosa Elena Mares Alejandre. Universidad Autónoma de Baja California |

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| **MV-63** | **Types of melanin produced in the grapevine phytopathogenic fungus *Lasiodiplodia theobromae.*** *Edelweiss Airam Rangel Montoya,* Marcos Paolinelli Alfonso, Rufina Hernández Martínez. Centro de Investigación Científica y de Educación Superior de Ensenada |
| **MV-64** | **Genetic and Antigenic relation of VP8* and VP5* subunits of VP4 protein of Rotavirus on Northern Mexico**. *César Iván Romo Sáenz,* Griselda Edith Menchaca Rodríguez, Roció Infante Ramírez, Licet Villarreal Treviño, Carlos Eduardo Hernández Luna, Cristina Rodríguez Padilla, Reyes Tamez Guerra, Juan Francisco Contreras Cordero. Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo Léon |
| **MV-65** | **Expression of HBD-2 in patients with viral infections and asthmatic crisis.** *Dora Patricia Rosete Olvera,* Carlos Cabello Gutiérrez, Ignacio Paramo Ramírez, Roció Chapela Mendoza, José Luis Sandoval Gutiérrez, Christian Trejo Jasso. Instituto Nacional de Enfermedades Respiratorias Ismael Cosio Villegas |
| **MV-66** | ***In Vitro* Evaluation of Antibacterial Activity of the Strain *Lactobacillus paracasei* KSI.** *Azarel Ruiz Román*, Laura Martínez Pérez, Ricardo Carrasco Torres, Patricia Aguilar Alonso, Gloria León Tello, Juan Carlos Benítez Serrano. Facultad de Cs. Químicas, BUAP |
| **MV-67** | **Analysis of transcriptional expression of f17 fimbriae and of the build of biofilm in *Gallibacterium anatis*.** *Ma. Patricia Sánchez Alonso,* Miguel Ávalos Rangel, Estela Anastacio Marcelino, Candelario Vázquez Cruz, Erasmo Negrete Abascal. Instituto de Ciencias. BUAP |
| **MV-68** | **Phenotypic analysis of *cdgC*::GusA-Sp$^{R}$ mutant of a diguanylate cyclase from *Azospirillum brasilense*sp 245.** *Daniel Sierra Cacho*, Alberto Ramírez Mata, María Luisa Xiqui Vázquez, Beatriz E. Baca. Instituto de Ciencias. BUAP |
| **MV-69** | **Microbial diversity in the intestinal tract of the Pacific reproductive geoduck clam Panopeaglobosa (Dall 1898).** *Hortencia Silva Jiménez,* Cynthia L. Araujo Palomares, Zaúl García Esquivel, Alma R. Corrales Escobosa, Katarzyna Wröbel, Tatiana N. Olivares Bañuelos. División de Biología Molecular, Universidad Autónoma de Baja California |
| **MV-70** | **Isolation and characterization of influenza A virus strains in Mexican patients**. *William Toledo Rueda,* Luis Márquez Domínguez, César González Bonilla, Esteban Muñoz Medina, Julio Reyes Leyva, Verónica Vallejo Ruiz, Nora Rosas Murrieta, Gerardo Santos López. Centro de Investigación Biomédica de Oriente. IMSS |
| **MV-71** | **Physiological characterization of conidia of *Sclerotium cepivorum* Berk: causal agent of garlic white rot disease.** *Jessica Edith Torres Granados,* Christian Lona Arrona, Ma. de Lourdes Palma Tirado, Alberto Flores Martínez, Patricia Ponce Noyola. Departamento de Biología, Universidad de Guanajuato |
| **MV-72** | **Multiple serum proteins as opsonins of *Sporothrix schenckiiconidia.*** Jazmín Sánchez Morales, Augusto González Canto, Alma Escalona Montaño, *Haydee Torres Guerrero*. Facultad de Medicina, UNAM |
| **MV-73** | **Metabolomic study of *Lasiodiplodia theobromae***. *Carla Uranga Solís,* Joris Beld, Anthony Mrse, Ivan Córdova Guerrero, Michael Burkart, Rufina Hernández Martínez. Centro de Investigación Científica y de Educación Superior de Ensenada |
| **MV-74** | **Cloning and expression of the ORFan g26 from coliphage mEp021.** *Guadalupe Valencia Toxqui,* Eva Martínez Peñafiel, Luis Kameyama. Departamento de Genética y Biología Molecular, CINVESTAV Zacatenco |
| **MV-75** | **Association of Human Papilloma Virus with prostate cancer in Mexican Men.** *Vania Alejandra Valenzuela Rodríguez*, María Olivia Medel Flores, María del Consuelo Gómez García, David Guillermo Pérez Ishiwara, Virginia Sánchez Monroy. ENMyH. Instituto Politécnico Nacional |
| **MV-76** | **Searching for cell envelope components involved in the coliphage mEp021 infection.** *Roxana Yessika Vargas Jerónimo,* Eva Martinez Peñafiel, Luis Yoshio Kameyame Kawabe. Department of Genetics and Molecular Biology, CINVESTAV Zacatenco |
| **MV-77** | **Influenza A virus down regulates the expression of thrombo modulin in endothelial cells.** *Pedro Vargas Leonor,* Fernando Hernández Sánchez, Guillermo Cordero García, Luis Ángel Pérez Moreno, Carlos Cabello Gutierrez. Instituto Nacional de Enfermedades Respiratorias Ismael Cosio Villegas |

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| **MV-78** | **Cloning and expression of genes encoding fimbrial proteins F17 of Flf3 operon from *Gallibacterium anatis*.** *José Pablo Velázquez Valtierra,* María Elena Cobos Justo, Erasmo Negrete Abascal, Candelario Vázquez Cruz, Ma. Patricia Georgina Sánchez Alonso, Norma Elena Rojas Ruiz. Instituto de Ciencias, Universidad Autónoma de Puebla |
| **MV-79** | **Conservation of cell polarity markers in the Kingdom Fungi.** *Jorge Verdín,* Robert W Roberson Meritxell Riquelme. Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, AC. |
| **MV-80** | **IL28B gene polymorphisms rs8099917 and rs12979860 with response to treatment with Pegylated Interferon and Ribavirin in patient swith Hepatitis C.** *Norberto Vibanco Pérez,* Ma. de Jesús Durán Avelar, Miriam G. Vázquez Herrera, Pedro Castro Melchor, J. Francisco Zambrano Zaragoza, Jaime Sánchez Meza, Zulia F. Nieves López, Victor A. Sánchez Chávez. Unidad Académicas de Ciencias Químicas, Universidad Autónona de Nayarit |
| **MV-81** | **Disruption of anion exchanger 1 (band-3) by plasmid-encoded toxin a serine protease from entero aggregative *Escherichia coli*.** *Jorge Mateo Villaseca Flores,* Patricia Villaseca Torres, David González Salado, Patricia Moreno Durán. Departamento de Microbiología y Parasitología. Facultad de Medicina. UNAM |
| **MV-82** | **TEA proteins in apical organization in *Neurospora crassa***. *Fausto M. Villavicencio Aguilar,* Rosa R. Mouriño Pérez. CICESE |

# *Friday*

Friday November 11, 2016

8:30 – 10:30 **Plenary Session 10**

San Marcos Room I

**Epigenetics**

Chair: *Mario Zurita*
Instituto de Biotecnología, UNAM

8:30 – 9:00 Mitotic bookmarking by TFIIH during zygotic genome activation in *Drosophila* suggests the existence of a short-term transcriptional memory mechanism
**Mario Zurita**, Mandy Juárez, Sarai ValerioCabrera and Grisel CruzBecerra
Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, UNAM

9:00 – 9:30 Genome-wide mapping of tissue-specific enhancers during Drosophila development
**Kasia Oktaba**
University of California at Berkeley. USA

9:30 – 10:00 The long-range promoter interactions landscape of pluripotent cells
**Mayra Furlan Magaril**
Instituto de Fisiología Celular, UNAM

10:00 – 10:30 The circadian clock: a biological system at the crossroad of metabolism and epigenetics
**Lorena Aguilar Arnal**, Paolo Sassone Corsi.
Instituto de Investigaciones Biomedicas, UNAM

8:30 – 10:30 **Plenary Session 11**

San Marcos Room II

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**Computational Biology**

Chair: *Cei Abreu Goodger*
LANGEBIO CINVESTAV Irapuato

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8:30 – 9:00 Extracellular small RNAs during parasite-host communication
**Cei Abreu Goodger**, Cesaré Ovando Vazquez, Franklin Chow, Georgios Koutsovoulos, Tuhin Maity, Mark Blaxter, Julie Claycomb, Amy Buck
LANGEBIO CINVESTAV Irapuato

9:00 – 9:30 A network approach to pharmaceutical resistance in breast cancer
**Enrique Hernández Lemus**, Guillermo de Anda Jáuregui, Jesús Espinal Enríquez Raúl A. MejíaPedroza
Computational Genomics Division. National Institute of Genomic Medicine

9:30 – 10:00 Inter-species comparison of endothelial cell gene regulation reveals the conserved control of vascular disease genes
**Alejandra Medina Rivera,** Lina Antounians, Azad Alizada, Michael Liang, Lan Dang, France Gagn
Genetics and Genome Biology, SickKids Research Institute. Laboratorio Internacional de Investigación sobre el Genoma Humano. UNAM

10:00 – 10:30 Microbial Motility and Random Walkers
**Moisés Santillán**
CINVESTAV IPN Unidad Monterrey

10:30 – 11:00 Coffee break
Foyer San Marcos Room

**Concurrent Sessions**

Friday November 11, 2016

| 11:00 – 13:30 | **13 Systems Biology & Bioinformatic**<br>**San Marcos One Room** | **14 Basic Biochemistry**<br>**San Marcos Two Room** | **15 Immunology & Parasitology**<br>**San Marcos Three Room** |
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| | Chair: *Alexander De Luna*<br>LANGEBIO CINVESTAV | Chair: *Alejandro Sosa*<br>Facultad de Medicina. UNAM | Chair: *Erik Sierra*<br>Univ. Juárez Edo. Durango |
| 11:00-11:20 | **Role of miRNAs in breast cancer regulatory networks.** *Diana Drago García*, Jesús Espinal Enríquez, Enrique Hernández Lemus. National Institute of Genomic Medicine | **Phylogenetic profile, sequence conservation and genetic complementation assays of the protein family EFL1.** *Alfonso Méndez Godoy*, Magali Honey Escandón, Nuria Sánchez Puig.Instituto de Química,UNAM | **Employing cactophilic *Drosophila* species to study metabolic diseases** *Daniel Cázarez García*, Mariana Ramírez Loustalot Laclette, Therese Ann Markow, Robert Winkler.CINVESTAV Irapuato |
| 11:20-11:40 | **MiRNOME Landscape Analysis Reveals a 30 miRNA Core in Retinoblastoma** *M. Verónica Ponce Castañeda*, Blanca Elena Castro Magdonel, Manuela Orjuela, Adda Jeanette García Chéquer, Ma. De Lourdes Cabrera Muñoz, Noé Durán Figueroa. IMSS | **Matrix metalloproteinase 13 (MMP13 or collagenase 3) play a key role during resolution of lung fibrosis.** *Sandra Cabrera Benítez*, Mariana Maciel Herrerías, Daniel Hernández Barrientos, Annie Pardo Cemo, Moisés Selman Lama. Facultad de Ciencias, UNAM | ***Trypanosoma cruzi:* Antigens identification of Mexican isolates.** *Teresa Itandehui Martínez Cuevas*, Alejandra Patricia Barranco Sosa, Luis Alberto Hernández Osorio, María del Carmen Guzmán Bracho and Rebeca Georgina Manning Cela. CINVESTAV Zacatenco |
| 11:40-12:00 | **Genomic reconstruction of the evolutionary history of *Phaseolus vulgaris:* from early speciation to recent domestication in America**. *Martha Rendón Anaya*, Josaphat M. Montero Vargas, Soledad Saburido Álvarez, Anna Vlasova, Salvador Capella Gutiérrez, José Juan Ordaz, Toni Gabaldón, Luis Delaye Arredondo, Paul Gepts, Robert Winkler, Roderic Guigó, Alfonso Delgado Salinas, Alfredo Herrera Estrella.LANGEBIO CINVESTAV Irapuato | **Identification of membrane proteins of *Helicobacter pylori,* which bind human haemoglobin.** *José de Jesús Olivares Trejo*, Cristhian Sánchez Cruz, Marco Antonio González López, Norma Velázquez Guadarrama. CONACYT Universidad Autónoma Metropolitana | **Erythrocyte Sialoglycoproteins Bind Siglec-9 to Maintain Neutrophil Quiescence in the Bloodstream** *Ismael Secundino*, Anel Lizcano Simón Döhrmann, Ross Corriden, Sandra Díaz, Lingquan Deng, Víctor Nizet, Ajit Varki. Instituto de Biotecnología, UNAM |
| 12:00-12:20 | **Computational modeling of TLR5 and TCR cooperation for adult and neonatal CD4 T cell activation.** *Otoniel Rodriguez Jorge*, Linda Aimara Kempis Calanis, Darely Yarazeth Gutiérrez Reyna, Oscar Ramirez Pliego, Wassim Abou Jaudé, Morgane Thomas Chollier, Salvatore Spicuglia, Maria Angélica Santana Calderón, Denis Thieffry. UAEM | **SMc03960 is a thioesterase from *Sinorhizobium meliloti* with broad substrate specificity capable of producing 2-tridecanone** *Geovanny Rivera Hernández*, Lydia M. Bernabéu Roda, Christian Sohlenkamp, Otto Geiger, María José Soto, Isabel M. López Lara.CCG UNAM | **The zinc-mediated 50 kDa-metalloproteinase, tvMP50 of *Trichomonas vaginalis* is involved in cytototoxic prostatic cells.** *Jonathan Puente Rivera*, José Luis Villalpando Aguilar, Alma Villalobos Osnaya, Laura Isabel Vázquez Carrillo, María Elizabeth Álvarez Sánchez. UACM |

| | **13 Systems Biology & Bioinformatic**<br><br>**San Marcos One Room** | **14 Basic Biochemistry**<br><br>**San Marcos Two Room** | **15 Immunology & Parasitology**<br>**San Marcos Three Room** |
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| | Chair: *Alexander De Luna*<br>LANGEBIO CINVESTAV | Chair: *Alejandro Sosa*.<br>Facultad de Medicina. UNAM | Chair: *Erik Sierra*.<br>Univ. Juárez Edo. Durango |
| 12:20-12:40 | **Early evolution of gene regulatory elements in the animal kingdom.**<br>*Selene L. Fernández Valverde*, Bernard M. Degnan.<br>LANGEBIO. CINVESTAV. Irapuato | **Expression profile of Hsp90 alpha and Hsp90 beta identifies a patients subgroup with renal cell carcinoma with lower survival.** *Romero Mandujano Aline* Kay, Nadia Rangel Gauna, Miguel Jiménez Ríos, Delia Pérez Montiel Gómez, David Cantú de León, José Díaz Chávez, Norma Bobadilla Sandoval, Luis Alonso Herrera Montalvo, Carlo César Cortés González. Instituto Nacional de Cancerología | **Effect of cola consumption on growth and insulin resistance in rats with nutritional imbalance.**<br>*José Hugo Zavala Sánchez*, Héctor Urquiza Marín, Madeline Hernández Rebollar, Francisco Bolaños Jiménez.<br>Instituto de Investigaciones Químico Biológicas, Morelia Michoacán. |
| 12:40-13:00 | **Microrna-204 impairs angiogenesis by targeting pro-angiogenic ANGPTL2 and CREB5 transcription factor in breast cancer.**<br>*Carlos Palma Flores,* Ali FloresPérez, Laurence A. Marchat, Sergio Rodríguez Cuevas, Jesús Chimal Monroy, César López Camarillo. Posgrado en Ciencias Genómicas, UACM | **Identification of druggable binding pockets in the molecular surface of the TATA binding protein from eukaryotic parasites.**<br>*José Ángel Santiago Terrones*, Carmen Nina Pastor Colón. Centro de Investigación en Dinámica Celular, Universidad Autónoma del Estado de Morelos | **Angiogenesis is controlled by miR-204 through dual targeting of pro-angiogenic ANGPT1 and TGFBR2 genes in breast cancer.** *José Ali Flores Pérez*, Laurence a. Marchat, Sergio Rodríguez Cuevas, Alfredo Hidalgo Miranda, Elena Arechaga, Mónica Sierra Martínez, María l. Streber, Carlos PalmaFlores, Miguel Fonseca Sánchez, Juan Antonio González, César López Camarillo. UACM |
| 13:00-13:20 | **Structure of the bacterial ATP synthase**<br>*Edgar Morales-Ríos*, Martin G. Montgomery, Andrew G. W. Leslie and John E. Walker Mitochondrial Biology Unit. Medical Research Council. Cambridge, UK. and CINVESTAV Zacatenco | **Impact of presumed CD49f antagonist in the stemness of breast cancer cells.** *Marco Velasco Velázquez*, Inés Velázquez Quesada, Andrea Rodríguez Moreno, Sandra Guerrero Rodríguez, Charmina Aguirre Alvarado, Ángel RuizMoreno, Aldo Segura Cabrera.Facultad de Medicina, UNAM | **Apoptosis-like mitochondrial membrane permeabilization in *Trypanosoma brucei.***<br>*David Pérez Morga*, Gilles Vanwalleghem, Fréďéric Fontaine, Laurence Lecordier, Patricia Tebabi, Anneke Kremer, Gabriela Schumann Burkard, Joachim Rassow, Isabel Roditi & Etienne Pays.<br>Laboratoire de Parasitologie Moléculaire, Université Libre de Bruxelles, Belgium |

13:30 – 15:30 Lunch

15:30 – 17:30 Poster Session 4

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| IP | IMMUNOLOGY AND PARASITOLOGY |
| NN | NEUROSCIENCES AND NEUROLOGY |
| TP | TOXICOLOGY AND PHARMACOLOGY |
| ST | SIGNAL TRANSDUCTION |

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17:30 – 18:30 **Closing Lecture**
Epigenetics and Onco-metabolites
**Jasper Rine**
California Institute of Quantitative Biosciences
Department of Molecular and Cellular Biology. U.C. Berkeley

Chair: *Irene Castaño*
IPICYT

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18:30 – 19:00 Final announcements and Closing Ceremony

21:00 Closing Dinner

## IMMUNOLOGY AND PARASITOLOGY

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| **IP-1** | **Zip14 role in regulating the redistribution of plasma zinc into liver during inflammation and their induction by signaling pathway Jak-Stat.** *Violeta Aburto Luna*, Samuel Treviño Mora, Diana Moroni González, Patricia Aguilar Alonso, Bertha Alicia León Chávez, Eduardo Miguel Brambila Colombres. Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla |
| **IP-2** | **Effect of testosterone on oxidative stress in blood and spleen of CBA/Ca mice infected with *P. berghei* ANKA.** *Jesús Aguilar Castro*, Martha Legorreta Herrera. FES Zaragoza, UNAM |
| **IP-3** | **Purification of $IgG_1$ and $IgG_2$ from hyperimmunized bovine against *Anaplasma marginale.*** *Itzel Amaro Estrada*, Eduardo Vergara Rivera, Jesús Francisco Preciado de la Torre, Rosa Estela Quiroz Castañeda, Mayra ElizethCobaxin Cárdenas, Sergio Darío Rodríguez Camarillo. Centro Nacional de Investigación Disciplinaria en Parasitología Veterinaria del Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias |
| **IP-4** | **Analysis of differential expression between strains of *Trypanosoma cruzi*, with high or low infectivity.** Antonio Campos Alberto, Martínez Cuevas Teresa Itandehui, Guzmán Bracho María del Carmen, Martínez Calvillo Santiago, Alejandre Aguilar Ricardo, Manning Cela Rebeca. ENCB-IPN |
| **IP-5** | **Clustering of Ig-like domains during CRTAM-NECL2 Interaction.** *Juan Carlos Barragán Gálvez*, Vianney Ortiz Navarrete. CINVESTAV Zacatenco |
| **IP-6** | **Effect of oestradiol on the mRNA expression of IFN-γ and TNF-α in CBA/Ca mice infected with *P. berghei* ANKA.** *Fidel Orlando Buendía González*, Martha Legorreta Herrera. Facultad de Estudios Superiores Zaragoza, UNAM |
| **IP-7** | ***Trypanosoma cruzi* RNA polymerase I: Characterization of the nuclear localization signal of subunit RPA31.** *Israel Felipe Canela Pérez*, Cevallos A. María, López Villaseñor Imelda, Hernández Roberto. Instituto de Investigaciones Biomédicas, UNAM |
| **IP-8** | **Characterization of the cyst formation *in vitro* of *Toxoplasma gondii* and study of the role of the cytoskeleton of the host cell.** *Kitzia Nohemí Castro Elizalde*, Pedro HernándezContreras, Mónica Mondragón Castelán, Sirenia González Pozos, Ricardo Mondragón Flores. CINVESTAV Zacatenco |
| **IP-9** | **ERM proteins of mosquito *Aedes* sp., important vector of human disease.** *Febe E. Cázares Raga*, Irving U. Martínez Vargas, David Mayorga Calleja, Abel Trujillo Ocampo, Rosa M. del Angel, Fidel de la C. Hernández Hernández. CINVESTAV. Zacatenco |
| **IP-10** | **Effect of tamoxifen on the oxidative stress in CBA/Ca mice infected with *Plasmodium berghei* ANKA**. *Luis Antonio Cervantes Candelas*, Martha Legorreta Herrera. Facultad de Estudios Superiores Zaragoza, UNAM |
| **IP-11** | **Parasitological study of streams and channels of the Rancheria La Cruz de Olcuatitán Nacajuca, Tabasco.** *Blanca Estela Contreras Pérez*, Lorena I. Acosta Pérez, Ma. Antonia Luna Gómez, Iris Ricardez Córdova. Universidad Juárez Autónoma de Tabasco |
| **IP-12** | **Identification and characterization of a phosphatase 2C of *Toxoplasma gondii* and its possible role in regulation of conoid extrusion.** *Rosalba Cruz Mirón*, Mondragón Castelan Mónica, Castro Elizalde Kitzia, Emmanuel Rios, Mondragón Flores Ricardo. CINVESTAVIPN. Zacatenco |
| **IP-13** | **Effectiveness of both plants: *Cymbopogoncitratus* and *Curcuma longa* as prophylactic antimalarial treatments in *Plasmodium berghei* ANKA infected CBA/Ca mice.** *Omar Fernández Rivera*, Martha Legorreta Herrera. Facultad de Estudios Superiores Zaragoza, UNAM |
| **IP-14** | **Development of a detection method of *Acanthamoeba castellanii* with gold nanoparticles.** *María de la Luz García Jaime*, Lérida Liss Flores Villavicencio, Gloria Barbosa, Juan Luis Pichardo Molina, Pablo Eduardo Cardoso Ávila, Julio César Villagómez Castro, Mineko Shibayama, Myrna Sabanero. Departamento de Biología, DCNE, Universidad de Guanajuato |
| **IP-15** | **Activity of podophyllotoxin type lignans from *Bursera fagaroides var. fagaroides* against *Giardia lamblia trophozoites.*** *Filiberto Gutiérrez Gutiérrez*, Ana María Puebla Pérez, Sirenia González Pozos, José Manuel Hernández Hernández, Laura Patricia Álvarez Berber, Araceli Castillo Romero. Departamento de Química, Universidad de Guadalajara |

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| **IP-16** | **Effect of adipokinetic hormons on the regulation of antimicrobial peptides in *Anopheles albimanus* Mosquito.** *Grecia Gabriela Hernández Díaz*, Guillermo Perales Ortiz, Alejandro Alvarado Delgado, Humberto Lanz Mendoza. CISEI. Instituto Nacional de la Salud Pública |
| **IP-17** | **Expression of *EhCP1* in the bacterial periplasm and effect of the redox environment on its structure and function.** *Ekaterina Jalomo Khayrova*, Alexis Zarahy Minchaca Acosta, Patricia Lilián Alejandra Muñoz Muñoz, Rosa Elena Mares Alejandre, Samuel Guillermo Meléndez López, Marco Antonio Ramos Ibarra. Facultad de Ciencias Químicas e Ingeniería, Universidad Autónoma de Baja California |
| **IP-18** | **Searching for a peptidic natural ligand of Aminopeptidase N/ CD13, a phagocytic receptor.** *Georgina Ivette Lopez Cortes*, Ortega Soto Enrique. Instituto de Investigaciones Biomédicas, UNAM |
| **IP-19** | **Cloning, expression, and characterization of the recombinant mitosomal inorganic pyrophosphatase from *Entamoeba histolytica* (*Eh*IPP) and homology-based prediction of its three-dimensional structure.** *Samuel Guillermo Melendez López*, Marco Antonio Ramos Ibarra, Rogelio Rodríguez Sotres. Facultad de Ciencias Químicas e Ingeniería, Universidad Autónoma de Baja Califormia |
| **IP-20** | **Gene silencing of an amebic protein disulfide isomerase (*Eh*PDI).** *Patricia Lilián Alejandra Muñoz Muñoz*, Rosa Elena Mares Alejandre, Samuel Guillermo Meléndez López, Alexei Fedorovish Licea Navarro, Marco Antonio Ramos Ibarra. Facultad de Ciencias Químicas e Ingeniería, Universidad Autónoma de Baja California |
| **IP-21** | **Profilin: Expression and identification of its ligands in *Trypanosoma cruzi.*** *Juan Felipe Osorio Méndez*, Rebeca Manning, Santiago MartÍnez, Roberto Hernández, Ana Maria Cevallos. Departamento de Biología Molecular y Biotecnología. UNAM |
| **IP-22** | **Characterization and detection of hydrolytic enzymes from *Toxoplasma gondii* RH strain in whole-cell extract and excretory/secretory products.** *Carlos Jorge Ramírez Flores*, Mónica Mondragón Castelán, Rossana Arroyo Verástegui, Guillermo Ávila Flores y Ricardo Mondragón Flores. CINVESTAV IPN |
| **IP-23** | **Assessment of the involvement of protein kinase Eh-GSK3 in adhesion and phagocytosis of *Entamoeba histolytica.*** *José Manuel Ramírez Merino*, Lorenza Pintor Capulin, Eduardo Castañeda Saucedo, Napoleon Navarro Tito, Cecilia González Calixto and Mercedes Calixto Gálvez. Universidad Autónoma de Guerrero |
| **IP-24** | **Two P2X1 receptor isoforms form heteromeric channels.** *Rodríguez Meléndez Jessica*, López López Cintya, Gómez Coronado K. Sarahí, Espinosa Luna Rosa, Barajas López Carlos. División de Biología Molecular, IPICYT |
| **IP-25** | **Participation of the humoral and cellular activity in hemolymph of crayfish *Cherax quadricarinatus* challenge with β-1,3 glucan.** *Yesenia Sánchez Salgado*, José Luis Sánchez Salgado, Ma. Concepción Agundis Mata, Alí Pereyra Morales, Luis Fernando Cruz García, Edgar Galindo Zenteno, Claudia Sierra Castillo. Laboratorio de Biología Celular, Facultad de Ciencias Biológicas, UAEM |
| **IP-26** | **Hsa from *Streptococcus gordonii* induces the priming of neutrophils.** *Ismael Secundino Velázquez*, Yvonne Rosenstein. Instituto de Biotecnología,UNAM |
| **IP-27** | **P2X receptors in human macrophages.** *Vargas Martínez Eydie M*, López Lopez Cintya, Portales Pérez Diana P, Espinosa Luna R, Miranda Morales Marcela, Barajas López. IPICYT |
| **IP-28** | **Effect of the VSP9B10A protein from *Giardia duodenalis* on the intestinal epithelium, using gerbils (*Meriones unguiculatus*) as experimental model of giardiasis.** *Yéssica Vázquez Cóbix*, Fonseca Liñán Rocío, Daniel D. HernándezCueto, Gómez Jiménez Luz María, Huerta Yépez Sara, Ortega Pierres M. Guadalupe. CINVESTAV. Zacatenco |
| **IP-29** | **Gene expression and cytotoxicity of *Trichomonas vaginalis* during interaction with prostatic cells mediated by $Zn^{2+}$.** Laura Isabel Vázquez Carrillo, Edgar Yebrán Villegas Vázquez, José Luis Villalpando, Mauricio Castañón Arreola, María Elizbeth Alvarez Sánchez. Posgrado de Ciencias Genómicas, UACM |
| **IP-30** | **Expression of actin 2, an actin variant of *Trypanosoma cruzi*, and identification of some of its ligands**. Andrea Cristina Vizcaíno Castillo, Rebeca Manning Cela, Roberto Hernández, Ana María Cevallos. Instituto de Investigaciones Biomédicas, UNAM |

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| **IP-31** | **Effect of a Recombinant Probiotic on Lung Inflammation in an Experimental Asthma Model.** *Daniel Cervantes García*, Pamela Gallegos Alcalá, Cristian Vargas García, Luis Miguel Haro Jr Cornejo, Mariela Jiménez Vargas, Alicia Hernández Mercado, Claudia Berenice Barrón García, María de Jesús Loera Arias, Odila Saucedo Cárdenas, Roberto Montes de Oca Luna, Eva María SalinasMiralles. Departamento de Microbiología, Centro de Ciencias Básicas, Universidad Autónoma de Aguascalientes |
| **IP-32** | **B cells respond via germinal centers to produce anti-lipid IgG antibodies.** *Claudia Albany Reséndiz Mora,* Carlos Wong Baeza, Alonso Rubén Tescucano Alonso, Sandra Sánchez Barbosa, Viridiana Galicia Galicia, Jessica Cervantes López, Carlos Wong Ramírez, María Isabel Baeza Ramírez. Departamento de Bioquímica, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional |

## NEUROSCIENCES AND NEUROLOGY

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| **NN-1** | **The Role of Autophagy During the Mouse Neural Tube Closure**. *Pilar Sarah Acevo Rodríguez***,** Diana Escalante Alcalde, Susana Castro Obregón. Instituto de Fisiología Celular, UNAM |
| **NN-2** | **Activation of hippocampal adult neurogenesis in response to a context-fear memory task after an excitotoxic focal dentate gyrus lesion.** *Andrea Aguilar Arredondo***,** Clorinda Arias Álvarez, Angélica Zepeda Rivera. Instituto de Investigaciones Biomédicas, UNAM |
| **NN-3** | **Sympathetic fat denervation improvement the metabolic alteration caused for Sleep restriction.** *Lucia Engracia Azuara Alvarez***,** Adrian Baez Ruiz, Nadia Saderi, Roberto Carlos Salgado Delgado. Facultad de Ciencias, Universidad Autónoma de San Luis Potosí |
| **NN-4** | **Nicotine effect in a Parkinson's disease model induced by human α-Synuclein and Synphilin expression in *Drosophila melanogaster*.** *Luis Angel Carvajal Oliveros,* Nancy Aslhey Pérez Arizmendi, René Hernández Vargas, Enrique Alejandro Reynaud Garza. Instituto de Biotecnología, UNAM |
| **NN-5** | ***In vitro* and *in vivo* neuroprotective activity of *scammonin* 1 and *tyrianthin* C isolated from root of Ipomoea *tyrianthina***. *José Manuel Castro García,* Lucero Valladares Cisneros, Juana Villeda Hernández, Ismael León Rivera, María Del Carmen Gutiérrez Villafuerte. CEIB.Universidad Autónoma del Estado de Morelos |
| **NN-6** | **Modulation of gene expression of the REST/NRSF Complex by TIME-RESTRICTED Feeding in a pharmacological seizure model.** *Edit Michelle Delgado Galán***,** Octavio Fabián Mercado Gómez y Rosalinda Guevara Guzmán. Facultad de Medicina, UNAM |
| **NN-7** | **The recombinant five disulfide-bonded spider toxin rOxyTx1 is an insecticide peptide that block calcium channels in cockroach dorsal unpaired median (DUM) neurons.** *Georgina Estrada***,** Gerardo Corzo, Anita O. Silva, Elba Villegas, Ernesto Otíz, Paulo Sergio Beirao. Centro de Investigación Científica de Yucatán A.C. |
| **NN-8** | **Malva parvifloraimmunomodulatoryeffect in a mouse model of Alzheimer´sdisease.** *Cristina E. Ramírez Serrano,* Jaime Totoriello García, Enrique Jiménez Ferrer, Maribel Herrera Ruíz, Alejandro ZamilpaAlvarez, Gustavo Pedraza and Leonor Pérez Martínez. Laboratorio de Neuroinmunobiología, Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología, UNAM |
| **NN-9** | **Isoforms of REST in vertebrados evolutionarily distant vertebrates**. *Evaristo Priego Adilene,* Liborio Bautista Amarilis, Valdés Flores Jesús, Napoleón Navarro Tito, Olga Lilia Garibay Cerdenares, Ortuño Pineda Carlos. Universidad Autónoma de Guerrero |
| **NN-10** | **Effect of Krill oil in the hippocampus of adult rats with seizures induced by pentylenetetrazole and febrile seizures at five postnatal days.** *Gloria Isabel Girón de la Cruz,* Fridha Viridiana Villalpando Vargas, Laura Guadalupe Medina Ceja and Leopoldo Flores Mancilla. Centro Universitario de Ciencias Biológicas y Agropecuarias. Universidad de Guadalajara |
| **NN-11** | **Characterization of TRPV1 glycosylation.** *Ameyalli Gómez Ilescas,* Alejandra Llorente Gil, Félix Sierra Ramírez, Tamara Rosenbaum, Sara L. Morales Lázaro. Instituto de Fisiología Celular, UNAM |

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| **NN-12** | **Glycyrrhizin ameliorates oxidative stress and inflammation in hippocampus and olfactory bulb in lithium/pilocarpine-induced status epilepticus in rats.** *Susana González Reyes,* Juan Jair Santillán Cigales, Angélica Saraí Jiménez Osorio, José Pedraza Chaverri, Rosalinda Guevara Guzmán. Facultad de Medicina, UNAM |
| **NN-13** | **Role of autophagy in the physiological aging of the brain and the establishment of a senescent phenotype**. *Elisa Gorostieta Salas,* Daniel Moreno Blas, Susana Castro Obregón. Instituto de Fisiología Celular, UNAM |
| **NN-14** | **Analysis of the 5-HT1A Receptor in Major Depressive Disorder in rat.** *Daniel Juárez Serrano,* Carlos Enrique Ochoa Velasco, Obdulia Vera López, Teresa Soledad Cid, Patricia Aguilar Alonso. Facultad de Ciencias Químicas, Universidad Autónoma de Puebla |
| **NN-15** | **Sigma 1 Receptor as a novel regulator of the TRPV1 ion channel.***Rebeca Juárez Contreras,* Miguel Ortíz Rentería, Felix Sierra Ramírez, Tamara Rosenbaum, Sara L Morales Lázaro. Instituto de Fisiología Celular, UNAM |
| **NN-16** | **Effect of chronic Krill oil diet on epileptiform activity induced in adult rats by Pentylenetetrazole and with febrile seizures at 5 postnatal days.***Adriana Marisol Lara Vazquez,* Fridha Viridiana Villalpando Vargas, Laura Guadalupe Medina Ceja, Leopoldo Flores Mancilla. Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara |
| **NN-17** | **Isoforms of REST in vertebrados evolutionarily distant vertebrates.** *Liborio Bautista Amarilis,* Evaristo Priego Adilene, Valdés Flores Jesús, Napoleón Navarro Tito, Olga Lilia Garibay Cerdenares, Ortuño Pineda Carlos. Universidad Autónoma de Guerrero |
| **NN-18** | **Effect of neonatal administration clomipramine (CMI) on expression of REα and REβ in dorsal raphe nucleus.***Ofelia Limón Morales,* Marcela Arteaga Silva, Marco Antonio Cerbón Cervantes, Herlinda Bonilla Jaime. Facultad de Química, UNAM |
| **NN-19** | **Evaluation of *Lopezia racemosa* extract with anti-inflammatory activity in a mouse model for the study of Alzheimer's disease.** *Wilver Montes Dorantes,* Carolina Abarca Camacho, Ma. Del Carmen Gutiérrez Villafuerte. Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos |
| **NN-20** | **Effect of a synthetic hypothalamic hormone on body composition and mRNA Expression of Ghrelin and lipoprotein lipase.** *Carlos Olvera Sandoval,* Quintanar Stephano Andrés, Betanzos Cabrera Gabriel, Quintanar Stephano José Luis. Centro de Ciencias Básicas. Universidad Autónoma de Aguascalientes |
| **NN-21** | **Novel regulators of TRPV1 ion channel: effects in pain and itch sensations.** *Miguel Ortíz Rentería,* Tamara Rosenbaum, Sara Luz Morales Lázaro. Instituto de Fisiología Celular, UNAM |
| **NN-22** | **Kinetics of serum proinflammatory cytokines in a murine model of Alzheimer´s disease.** *María de la Luz Pérez Uscanga,* Luis Manuel Castillo, Priscila Judith Torres Granados, Luis Felipe Montaño Estrada, Erika Patricia Rendón Huerta. Facultad de Medicina, UNAM |
| **NN-23** | **Analysis of cytoskeletal proteins from spinal cord neurons of rat embryo treated with gonadotropin releasing hormone (GnRH).** *José Luis Quintanar Stephano,* Denisse Calderón Vallejo, Irma Hernández Jasso. Centro de Ciencias Básicas, Universidad Autónoma de Aguascalientes |
| **NN-24** | **Abnormal expression of matrix metalloproteases 9 and 2 in individuals with Alzheimer's disease.** *Luis Abraham Rosales García,* Mario Hernándes Alejandro, José Luna Muñoz, José Eduardo Pérez Salazar, Pedro Cortés Reynosa. Instituto Politécnico Nacional |
| **NN-25** | **Role of enteric glia in mesenteric afferent nerve activity.** *Ruiz Velarde Gabriela,* Villalobos Hernández E. Criseida, Ochoa Cortés Fernando, Christofi Ferias Leontin, Espinosa Luna Rosa, Barajas López Carlos, Miranda Morales Marcela. Instituto Potosino de Investigación Científica y Tecnológica A.C. |
| **NN-26** | **STIM and Orai as molecular biomarkers in calcium regulation in Alzheimer's disease.** *Andres Salas Casas,* Ana Laura Cano Martínez, Mario Alberto Rodríguez Rodríguez, José Luna Muñoz, Irma Zúñiga Hernández, Ana Hilda Figueroa Gutiérrez, David López Romero. Universidad Autónoma del Estado de Hidalgo |

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| **NN-27** | **Role of reactive oxygen species in palmitic acid-induced neuronal insulin resistance.** *Karina Sánchez Alegría,* Patricia Ferrera, Clorinda Arias. Instituto de Investigaciones Biomédicas, UNAM |
| **NN-28** | **Time-restricted feeding exerts anti-inflammatory and neuroprotective effects on acute seizure model.** *Juan Jair Santillán Cigales,* Jorge Landgrave Gómez, Octavio Fabián Mercado Gómez, Rosalinda Guevara Guzmán. Facultad de Medicina, UNAM |
| **NN-29** | **Effect of Taurine in the Progenitor-like Cell Generation from Müller Glia of mice.** *Carlos de Jesús Torres Rosas,* Rubén Zamora, Luis Fernando Hernández Zimbrón, Hugo Quiroz, Lenin David Ochoa De la Paz. Facultad de Medicina, UNAM |
| **NN-30** | **Functional expression of metabotropic glutamate receptors in the vestibular system of chicken embryo.** *Ricardo Varela Rodriguez,* Eduardo Monjaraz, Jorge Cebada, Amira Flores. Universidad Autónoma de Puebla |
| **NN-31** | **Effect of prolactin on microglial activation and expression of proinflammatory cytokines in the hippocampus of male rat pups.** *Guadalupe Zinzun Ixta,* Alejandra Ochoa Zarzosa, María de la Luz Torner Aguilar. Universidad Michoacana de San Nicolas de Hidalgo |

## TOXICOLOGY AND PHARMACOLOGY

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| **TP-1** | **Evaluation Of The Anti-inflammatory Capacity Of Methanol Extracts Of Wild Plants In Southern Sonora.** *Luis Alberto Zamora Alvarez,* Edgar Felipe Moran Palacio, Keisy Gabriela Velderrain Díaz, Norma Patricia Adan Bante, José Guadalupe Soñanez Organis. Universidad de Sonora |
| **TP-2** | ***In vivo* parameters in a model of type 2 diabetes mellitus in rats treated with pioglitazone.** *Samuel Álvarez Almazán,* Feliciano Tamay Cach, Diana Alemán González Duhart, Gustavo Guevara Balcázar, Jessica Elena Mendieta Wejebe. Escuela Superior de Medicina, IPN |
| **TP-3** | **Effect of a novel thiazolidinedione derived analogue on *in vitro* insulin-like response.** *Anaguiven Avalos Soriano,* Diana Alemán González Duhart, Jessica Elena Mendieta Wejebe. Escuela Superior de Medicina, IPN |
| **TP-4** | ***CYP2C9*3* Genetic Variant Is Independently Associated with Glycemic Control in Type 2 Diabetes Patients Treated with Glibenclamide.** *Osvaldo Daniel Castelán Martínez,* Carlos Hoyo Vadillo, Miguel Cruz, Tania Berenice Bazán Soto, Adán Valladares Salgado. FES Zaragoza, UNAM |
| **TP-5** | **Characterising the toxic activity profile of *Caybdea marsupialis* venom.** *Miguel Cuevas Cruz,* Ulises Hernández Guzmán, Judith Sánchez Rodríguez, Francisco Miguel Garcia Guerrero y Guerrero, Michelle Palacios Velasco, Viviana Edith Romero Cortés, José Fernando Lazcano Pérez, Roberto Arreguín Espinosa de los Monteros, Barbarín Arreguín Lozano. Instituto de Química, UNAM |
| **TP-6** | **A novel therapy for breast cancer triple negative mediated tumor suppression through mTOR inhibition and glycolysis aerobic.** *Izamary Delgado Waldo,* Veronica García Castillo, Eduardo López Urrutia, Nadia Jacobo Herrera, Carlos Pérez Plasencia. FES Iztacala, UNAM |
| **TP-7** | **Molecular analysis of cell death type induced therapy coadyuvant: doxorubicin-metformin and sodium oxamate as inhibitors of the mTOR pathway and glycolysis cell model in a triple negative breast cancer.** *Verónica García Castillo,* Nadia J. Jacobo Herrera, Eduardo López Urrutia, Luis E. Gómez Quiroz, Carlos Pérez Plasencia. FES Iztacala, UNAM |
| **TP-8** | **Analysis of the presence of adducts of Benzo [a] pyrene in the DNA of placental tissue as a measure to environmental genotoxic compounds exposure during pregnancy.** *Maria del Carmen Garcia de Leon Mendez,* Marie O'Neill, Jorge Beltrán Montoya, Vanesa Morales, Violeta Castro Leyva, Erika Chavira Suarez, Felipe Vadillo Ortega. Facultad de Medicina, UNAM |
| **TP-9** | **Role of angiotensin II in adrenergic vascular contraction during Diabetes Mellitus.** *Danny Peniel García Treviño,* Asdrúbal Aguilera Méndez, Zurisaddai Hernández Gallegos, Daniel Godínez Hernández. Michoacana de San Nicolás de Hidalgo |
| **TP-10** | **Detection of galphimines through TLC, HPLC, and $^{1}$H NMR in natural populations of the Mexican plant *Galphimiaglauca* Cav. (Malpighiaceae).** *Reinier Gesto Borroto,* Jan Schripsema, Alexandre Cardoso Taketa, María Luisa Villarreal Ortega. Universidad Autónoma del Estado de Morelos |

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| **TP-11** | **Pinocembrin ameliorates metabolic disturbances of diabetic nephropathy.** *Jessica Granados Pineda,* J. Fausto Rivero Cruz, Jazmin PérezRojas. Instituto Nacional de Cancerología |
| **TP-12** | **Antioxidant and antimicrobial activity of *Costuspulverulentus* extracts: a plant used in HuastecaPotosina traditional medicine.** *Angel León Buitimea,* Asdrúbal Flores Montoya, Vicente Olvera González, Brenda Alvarado Sánchez, Abigail Reyes Mungía. Universidad Autónoma de San Luis Potosí |
| **TP-13** | **Neurotoxic effects of early exposure to the herbicide atrazine during the development of zebrafish (*Danio rerio*).** *Mayra López Cervantes,* Selma Torres Valles, Everardo Gutiérrez, Verónica Rodríguez Córdova, Ulises Bardullas. Universidad Autónoma de Baja California |
| **TP-14** | **Hepatoprotective effect of *Callistemon citrinus* on paracetamol induced liver toxicity in rats.** *Alejandro López Mejía,* Patricia Ríos Chávez, Daniel Godínez Hernández, Blanca Nateras Marín. Universidad Michoacana de San Nicolas de Hidalgo |
| **TP-15** | **Peptide toxins modulate progesterone-induced $Ca^{2+}$ influx through CatSper ion channel in human sperm.** *Arlet del Carmen Loza Huerta,* Elizabeth Cervantes Ibarra, Héctor Cardoso, Alejandro Alagón, LourivalPossani, Arturo Hernández, Alberto Darszon, Arturo Picones Medina. Instituto de Fisiología Celular, UNAM |
| **TP-16** | **Melatonin efffect on lead bio-distribution.** *Minerva Martínez Alfaro,* Yolanda Alcaraz Contreras, Juvencio Robles, Alberto Flores, Marco Antonio García, Karla Soto Arredondo, Erick Díaz Cervantes. Universidad de Guanajuato |
| **TP-17** | **Antitumor activity of extract of *Ficuscrocata* in breast cancer cells MDA-MB-231.***Miguel Angel Mendoza Catalán,* Jhonathan Uriel Castillo Reyes, Gabriela García Prudente, Karina Melquiades Campos, Patricia Álvarez Fitz, Carlos Ortuño Pineda, Napoleón Navarro Tito, Marco Antonio Leyva Vázquez, Ana Elvira Zacapala Gómez. Universidad Autónoma de Guerrero |
| **TP-18** | **Perinatal administration of bisphenol A alters the expression of tight junction proteins in the uterus and reduces the implantation rate.** *Annia A. Martínez Peña*, Jorge Rivera, Marcos Ramírez, Laura Méndez, Aarón Galindo, J. Carlos Páez Franco, SumikoMorimoto, Lorenza González Mariscal, M. Esther Cruz,C. Adriana Mendoza Rodríguez.UNAM |
| **TP-19** | **Differential expression of drug transporters associated with chemoresistance in soft tissue sarcomas from child: Comparison between tumor and normal tissue.** *Dora Molina Ortiz,* Carmen Torres Zárate, Araceli Vences Mejía, José Martín Palacios Acosta, Daniel Hernández Arrazola, Rocío Cárdenas Cardós, Jaime Shalkow Kalincovstein. Instituto Nacional de Pediatría |
| **TP-20** | **Antinflamatory activity of isolated sesquiterpenic compounds from *P. decompositum*.** *Beatriz Mora Ramiro,* Rosario Wendoline Rosiles Alanis, Rosario Tavera Hernández, Francisco Javier Alarcón Aguilar, Manuel Jiménez Estrada, Julio Cesar Almanza Pérez. Universidad Autónoma Metropolitana Unidad Iztapalapa |
| **TP-21** | **Bioguided isolation of antifungical compounds form *Zizyphus obtusifolia*.** *Edgar Felipe Moran Palacio,* Luis Alberto Zamora Alvarez, Rossana Guadalupe GuiradoChávez, Guadalupe González Ochoa, Ramona IcedoGarcía. Universidad de Sonora |
| **TP-22** | **Evaluation of antioxidant activity of *Callistemon citrinus* on subacute oral toxicity in male Wistar rats.** *Patricia Rios Chávez,* Salvador Pérez Mora, Alejandro López Mejía, Daniel Godínez Hernández, Blanca Nateras Marin. Universidad Michoacana de San Nicolas de Hidalgo |
| **TP-23** | **Biotin supplementation has no detrimental effects on oxidative stress markers but induces genotoxicity in mice.** *Leticia Riverón Negrete,* Sicilia Argumedo Gloria, Álvarez Delgado Carolina, Fernández Mejía Cristina. Unidad de Genética de la Nutrición, INP. IIB, UNAM |
| **TP-24** | **Evaluation of the antimicrobial activity of short variants of arachnid antimicrobial peptides.** *Alexis J. Rodríguez Solis,* Elba C. Villegas Villarreal, Gerardo A. Corzo Burguete, María Belem Jiménez Huicochea, Johana Martínez Paula. Universidad Autónoma del Estado de Morelos |
| **TP-25** | **Cytotoxic activity by a peptide from the venom of the centipede *scolopendra polymorpha* in the HeLa cell line.** *Arturo Ronces Alvarado,* María del Carmen Gutiérrez Villafuerte. Universidad Autónoma del Estado de Morelos |

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| **TP-26** | ***Cucurbita Ficifolia*Bouché effects over energetic balance and inflammatory mediators on co-cultured adipocytes and macrophages**. *Wendoline Rosiles Alanis,* Julio Almanza Pérez, Ángeles Fortis Barrera, Alejandro Zamilpa Álvarez, Beatriz Mora Ramiro, Francisco Javier Alarcón Aguilar. Facultad de Ingeniería Ambiental, Universidad Autónoma Metropolitana Unidad Iztapalapa |
| **TP-27** | **Preclinical screening of CD44 antagonist in breast cancer cells.** *Angel Ruiz Moreno,* Charmina Aguirre Alvarado, Aldo Segura Cabrera, Marco Velasco Velázquez. Facultad de Medicina, UNAM |
| **TP-28** | **Cadmium exposure produces insulin resistance, impaired insulin signaling and steatosis in liver of Wistar rats.** *Victor Enrique Sarmiento Ortega,* Eduardo Brambila, José Ángel Flores Hernández, Patricia Aguilar Alonso, Diana Moroni, Alfonso Díaz, Samuel Treviño. Facultad de Ciencias Químicas, Universidad Autónoma de Puebla |
| **TP-29** | **Two antidepressants with different mechanisms reduce behavioral deficits in a rat model of major depression.** *Jocelyn Soto Esquivel,* Evoli López Morán, Eduardo Miguel Brambila Colombres, Julio César Morales Medina, Patricia Aguilar Alonso. Benemérita Universidad Autónoma de Puebla |
| **TP-30** | **Venom fractions of *Scolopendra polymorpha* with myotoxic activity on mice**. *Judith Tabullo De Robles,* Francisca Fernández Valverde, Lucero Valladares Cisneros, Juana Villeda Hernández, Ma. Del Carmen Gutiérrez Villafuerte. Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos |
| **TP-31** | **Implementation of an inexpensive system to evaluate the effect of environmental contaminants on motor behavior in zebrafish (*Danio rerio*).** *Selma Sofia Torres Valles,* Everardo Gutiérrez López, Ulises Pacheco Bardullas. Facultad de Ciencias, Universidad Autónoma de Baja California |
| **TP-32** | **Protective effect of Theophylline in aorta versus toxic effect of $T_iO_2$ in wistar rats.** *Francisco Moisés Treviño González,* Rosa María Chávez Morales, Salvador Acevedo Martínez, Ma. Consolación Martínez Saldaña. Universidad Autónoma de Aguascalientes |

## SIGNAL TRANSDUCTION

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| **ST-1** | **Logical model of the Wnt/B-catenin pathway in neonatal and adult human $CD8^+$ T cells**. *Oscar Bruno Aguilar Luviano*, Otoniel Rodríguez Jorge, Gerson Ney Hernández Acevedo, María Angélica Santana Calderón. Centro de Investigación en Dinámica Celular, Universidad Autónoma del Estado de Morelos |
| **ST-2** | **Zinc role in mouse spermatogenic cells.** *Karla Lisette Andrade López*, Paulina Torres Rodríguez, Ignacio López González, Julio César Chávez Zamora, Claudia Lydia Treviño Santa Cruz. Instituto de Biotecnología, UNAM |
| **ST-3** | **Primary Hepatocytes Undergo an Epithelial to Mesenchymal Transition in Response to TGF-beta Signaling.** *Yuli Aranda López*, Diana G. Ríos López, Marcela Sosa Garrocho, Marina MacíasSilva. Instituto de Fisiología celular, UNAM |
| **ST-4** | **Identification of microRNAs Modulated During Osteoblastic Differentiation of Human Mesenchymal Stem Cells ($CD105^-$).** *Mariana Melisa Avendaño Félix*, Lizeth Fuentes Mera, Vanessa Pérez Silos, Nidia Moncada Saucedo, Selem Tórres Méndez, Maribel Aguilar Medina, Rosalío Ramos Payán, Laurence A. Marchat, César López Camarillo. Laboratorio de Inmunología y Microbiología. Facultad de Ciencias Químico Biológicas, Culiacán, Sinaloa |
| **ST-5** | **The cancer associated Gpn3-Q279* mutant is defective to support RNA polymerase II nuclear targeting.** *Angel Adán Barbosa Camacho*, Lucía E. Méndez Hernández, Bárbara Lara Chacón, Sonia G. Peña Gómez, Violeta Romero, José A. Guerra Moreno, Rogelio González González, Angélica Y. Robledo Rivera, Roberto Sánchez Olea, Mónica R. Calera. Instituto de Física, Universidad Autónoma de San Luis Potosí |
| **ST-6** | **Effect of overexpression of dystroglycan on the physiology of HL-60 cells.** *Tizziani Benitez Guerrero*, Ivette Martínez Vieyra, Lea Alonso Rangel, Bulmaro Cisneros Vega, Doris Atenea Cerecedo Mercado. Escuela Nacional de Medicina y Homeopatía, Instituto Politécnico Nacional |

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| **ST-7** | **$Ca^{2+}$ Signal Evoked by Histamine in Normal Human Lung Fibroblasts.** *Roberto Berra Romani*, José Alonso Romero, Francesco Moccia, Franco Tanzi, Luis Guillermo Vázquez de Lara. Facultad de Medicina, Benemérita Universidad Autónoma de Puebla |
| **ST-8** | **Evaluation of treatment of second degree burns with adipose derived mesenchymal stem cell using human radiosterilized amnion and pig skin as scaffolds**. *Beatriz del Carmen Cabello Arista*, Martínez Pardo María, Velasquillo Martínez Cristina, Sánchez Sánchez Roberto. Instituto Nacional de Rehabilitación |
| **ST-9** | **The role of MAPKKK Ste11 in stress responses of *Trichoderma atroviride***. *Gabriela Calcáneo Hernández*, Fidel Landeros Jaime, José Antonio Cervantes Chávez, Alfredo Herrera Estrella, Edgardo Ulises Esquivel Naranjo. Universidad Autónoma de Querétaro |
| **ST-10** | **Effect of hydrogen sulfide on yeast autophagy.** *Anaid Alethia Cándido López*, Paola Moreno Álvarez Francisco Torres Quiroz. Instituto de Fisiología Celular, UNAM |
| **ST-11** | **Analysis of of UAP56 homologue (RNA helicase) participation in the mRNA export of C6/36 cell line derived from *Aedes albopictus.*** *Antonio Celestino Montes*, Abel Trujillo Ocampo, José Ángel Rubio Miranda, Fernando Medina Ramírez, Leticia Cortés Martínez, Febe Elena Cazares Raga, Rosa María Del Ángel, Fidel de la Cruz Hernández Hernández. CINVESTAV Zacatenco |
| **ST-12** | **Estrogen-mediated down-regulation of E-cadherin in breast cancer cells is mediated by c-Src and promotes cell migration and invasion**. *Fabiola Córdova Gerón*, Baní Isaí Menor Calderón, Carlos Lara Cruz, Pablo Damián Matsumura, Javier Esteban Jiménez Salazar. UAMI |
| **ST-13** | ***PKCz-CDP-FIH-HIF* status at early stages of renal carcinogenesis and tumors induced by ferric nitrilotriacetate.** *Patricia Curiel Muñiz*, José Solano, Francisco AguilarAlonso, Telma Pariente Pérez, María Elena Ibarra Rubio. Facultad de Química, UNAM |
| **ST-14** | **Invadopodia formation and matrix metalloprotease secretion during epithelial-mesenchymal transition induced by leptin in MCF10A epitelial cells.** *Jose Luis Dena Beltrán*, Miriam Daniela Zuñiga Eulogio, Miguel Ángel Mendoza Catalán, Sócrates Villegas Comonfort, Fernando Candanedo González, Napoleón Navarro Tito. Facultad de Ciencias Quimico Biologicas, Universidad Autónoma de Guerrero |
| **ST-15** | **Estudio de la fosfolipasa D en los mecanismos de migración e invasión inducidos por ácido linoleico en células de cáncer de mama MDA-MB-231.** *Ricardo Díaz Aragon*, Nathalia Serna Marquez, Alejandra Paola García Hernández, José Eduardo Pérez Salazar. CINVESTAV Zacatenco |
| **ST-16** | **Characterization of protein complexes associated to the phosphatidy linositol 3-kinase during *Phaseolus vulgaris* rhizobia interaction.** Claudia V. Dorantes Torres, Georgina Estrada Navarrete, Rosario Vera, Carmen Quinto, Federico Sánchez †. Instituto de Biotecnología, UNAM |
| **ST-17** | **The effect of hyperglycemia on the expression of stress proteins of endoplasmic reticulum and vascular smooth muscle cell (VSMC) migration fron normal rats.** *Ricardo Espinosa Tanguma*, Rebeca Mejía Elizondo, Aurelio Hernández Méndez. Facultad de Medicina, Universidad Autónoma de San Luis Potosí |
| **ST-18** | **Non-Classical Effects of Androgens in Muscle Cells.** *Dennys Paola Ferreyra Picazo*, Jesús Alberto Olivares Reyes. CINVESTAV Zacatenco |
| **ST-19** | **Isolation, characterization and neural differentiation of Wharton´s Jelly Mesenchymal Stem Cells from Human Umbilical Cord.** *Flores Reyes Josiff Samuel*, Oscar Pérez Pérez, Sandra R. Cruz Bárcenas, Ismael Mancilla Herrera, Mauricio Domínguez Castro, Higinio Estrada Juárez, Mónica Aguinaga Rios, Enrique Reyes Muñoz, José Romo Yáñez. Departamento de Genómica Humana, Instituto Nacional de Perinatologia |
| **ST-20** | **Apigenin regulates the expression of inflammatory kinases induced by LPS of *Porphyromonas gingivalis* in cell line H9c2.** *Flores Sánchez Mónica Arisbet*, Gloria Gutiérrez Venegas. Departamento de Investigación en Posgrado. Facultad de Odontología, UNAM |
| **ST-21** | **Effects of p-Chloroamphetamine (pCA) on Follicular Development and Apoptosis in Prepuberal Rat**. *Magaly Garcia Galicia*, Andrés Aragón Martínez, Maribel Flores Flores, María Elena Ayala Escobar. FES Zaragoza, UNAM |

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| **ST-22** | **Native type I collagen induces an epithelial to mesenchymal transition process in mammary epithelial cells MCF10A.** *Alejandra Paola Garcia Hernández*, Ricardo Diaz Aragon, Pedro Cortes Reynosa Eduardo Perez Salazar. CINVESTAV Zacatenco |
| **ST-23** | **Regulatory Mechanisms of Tumor Endothelium Marker 4 (ARHGEF17).** *Irving García Jiménez*, Daniel Cervantes Villagrana, Alejandro Castillo Kauil, Sendi Rafael Adame García, Víctor M. Color Aparicio, Guadalupe Reyes Cruz, José Vázquez Prado. CINVESTAV Zacatenco |
| **ST-24** | **The effect of hydrogen sulfide in the transcriptome of *Saccharomyces cerevisiae.*** *Cynthia García Nieves*, Francisco Torres Quiroz. Instituto de Fisiología Celular, UNAM |
| **ST-25** | **Role of TOR signaling pathway during *Azospirillum-Arabidopsis* interaction.** Manuel Méndez Gómez,Elda Castro Mercado, *Ernesto García Pineda*. Universidad Michoacana de San Nicolas de Hidalgo |
| **ST-26** | **Generation of Reporter Lines for the Study of MPK6 Role in the Development of Root Hairs in *Arabidopsis thaliana.*** *Isabel García Torres*, León Patricia, Guevara García Ángel Arturo, López Bucio Jesús Salvador. Instituto de Biotecnología, UNAM |
| **ST-27** | **c-Src inhibitor induces cell migration and invasion through the estrogen-mediated pathway in a Triple-Negative Breast Cancer cell line.** *Rosa Icela García Vázquez*, Isaí Bani Menor Calderón, Carlos Lara Cruz, Pablo Damián Matsumura, Javier Esteban Jiménez Salazar. Universidad Autónoma Metropolitana |
| **ST-28** | **GRP78 and ATF6 expression in low-high grade intraepithelial neoplasia and cervical cancer produced by HPV16.** *Alma Delia Hurtado Mercado,* Rafael Gutiérrez Campos. Universidad Autónoma de Aguascalientes |
| **ST-29** | **Does *Fagopyrum esculentum* encode an ABC transporter involved in Aluminum tolerance?** Gutiérrez Granados Natalia, Cruz Ortega Rocío. Instituto de Ecología, UNAM |
| **ST-30** | **Function of the peroxisome ubiquitination complex in the sexual development of the fungus *Podospora anserine*.** *Claudia Zirión Martínez*, Fernando Suaste Olmos, Leonardo Peraza Reyes. Instituto de Fisiología Celular, UNAM |
| **ST-31** | **Activation of protein kinase C promotes $\alpha_{1B}$-adrenergic receptor late-endosome trafficking through Rab-9 interaction.** *David A. Hernández Espinosa*, Marco A Alfonzo Méndez, Gabriel Carmona Rosas, M. Teresa Romero Ávila, Guadalupe Reyes Cruz, J. Adolfo García Sáinz. Instituto de Fisiología Celular, UNAM |
| **ST-32** | **Analysis of the expression and subcellular localization of Retinoblastoma mutants**. *Jesús Hernández Monge*, Adriana B. Rousset R., Vanesa Olivares Illana. Instituto de Física, Universidad Autónoma de San Luis Potosí |
| **ST-33** | **Expression of the Receptor for Activated C Kinase 1 in the photosynthetic dinoflagellate *Symbiodinium* during light/dark and growth phases**. *Tania T Islas Flores*, Esmeralda Pérez Cervantes, Jessica Nava Galeana, Gabriel Guillén, Marco A. Villanueva Méndez. Unidad Académica de Sistemas Arrecifales, UNAM |
| **ST-34** | **Phosphorylation of maize ribosomal proteins is stimulated by ZmIGF during germination.** *Laura Vanessa Jiménez Pérez*, Lilia Angélica Bernal Gracida, Estela Sánchez Quintanar. Facultad de Química, UNAM |
| **ST-35** | **Leptin induces epithelial-mesenchymal transition in a Src-dependent pathway in mammary epithelial cells.** *Juan Carlos Juárez Cruz*, Eduardo Castañeda Saucedo, Miguel A. Mendoza Catalán, Carlos Ortuño Pineda, Raúl Martínez Orozco, Napoleón Navarro Tito. Facultad de Ciencias Quimico Biologicas, Universidad Autónoma de Guerrero |
| **ST-36** | **Importance of PKCθ activity in the balance of activation of transcription factors AP-1, NFAT and NF-kB in human CD4 T lymphocytes and its implication on neonatal immunity.** *Linda Aimara Kempis Calanis*, Otoniel Rodríguez Jorge, María Angélica Santana Calderón. Centro de Investigación en Dinamica celular, Universidad Autónoma del Estado de Morelos |
| **ST-37** | **Effect of 100 IU of IL-2 on the proliferation of cervical carcinoma line HeLa and INBL.** *María del Carmen Lagunas Cruz*, Isabel Soto Cruz, Arturo Valle Mendiola, Benny Weiss Steider. FES Zaragoza, UNAM |

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| **ST-38** | **GTPases Gpn1 and Gpn3 regulate nuclear entry of Rpb1, the largest subunit of RNA polymerase II, as a protein complex.** *Bárbara Lara Chacón*, Mónica R. Calera and Roberto Sánchez Olea. Instituto de Física, Universidad Autónoma de San Luis Potosí |
| **ST-39** | **Over-expression of Myelin and Lymphocyte Protein (MAL) reduces MUC1-C oncoprotein trafficking to the nucleus.** *Antonio Roberto Lara Lemus*, Mónica Flores Torres, Ana Karina Saldaña Villa. Instituto Nacional de Enfermedades Respiratorias Ismael Cosio Villegas |
| **ST-40** | **Extracellular vesicles from MDA-MB-231 breast cancer cells stimulated with linoleic acid promote migration through PI3K/Akt signaling pathway in MCF10A cells.** *Elizabeth Leal Orta*, Octavio Galindo Hernandez, Javier Ramirez Ricardo, Eduardo Perez Salazar. Departamento de Biología Celular, CINVESTAV Zacatenco |
| **ST-41** | **Identification of the phosphorylated proteins by the Protein Kinase C during macrophage infection with *M. bovis.*** *Rafael Maldonado Bravo*, Edgardo Madrid Paulino, Tomás Villaseñor Toledo, Leonor Pérez Martínez, Gustavo Pedraza Alva. Instituto de Biotecnología, UNAM |
| **ST-42** | **Participation of ERK and Kpna2 in the nuclear translocation of Rac1 in HaCaT cells in response to estrogens.** *Angélica Martínez López*, Estefanía Yoceli Ojeda Muñiz, Patricia Garibo Nogeda, Mercedes Calixto Gálvez, Miguel A. Mendoza Catalán, Napoleón Navarro Tito, Eduardo Castañeda Saucedo. Laboratorio de Biología Celular del Cáncer, Universidad Autónoma de Guerrero |
| **ST-43** | **A New MDM2-MDMX heterodimer independent of C-terminal RING-RING interaction induced by ATM.** *Ixaura Medina Medina*, Vanesa Olivares Illana. Instituto de Física, Universidad Autónoma de San Luis Potosí |
| **ST-44** | **Characterization of a novel family of proteins involved in cholesterol transport.** *Kevin Manuel Méndez Acevedo*, Julián Valdés, Alexander Asanov, Luis Vaca. Instituto de Fisiología Celular, UNAM |
| **ST-45** | **Role of TOR signaling pathway during *Azospirillum-Arabidopsis* interaction.** *Manuel Méndez Gómez*, Ernesto García Pineda, Elda Castro Mercado. Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolas de Hidalgo |
| **ST-46** | ***EXPANSIN* gene family in *Lotus japonicus*: Identification, characterisation and expression analysis in response to symbiosis.** *Sudipta Kumar Mohanty*, Manoj Kumar Arthikala, Kalpana Nanjareddy and Miguel Lara. Instituto de Biología, UNAM |
| **ST-47** | **Effect of biotin on adipocyte differentiation.** *Ericka Moreno Méndez*, Cristina Fernández Mejía. Unidad de Genética de la Nutrición. UNAM |
| **ST-48** | **The expression of NR4A receptors and autophagy as part of the DNA damage response.** *Gabriel Muciño Hernández*, Daniel Moreno Blas, Susana Castro Obregón. Instituto de Fisiología Celular, UNAM |
| **ST-49** | **Role of Ser/Thr or Tyr protein kinase phosphatases on MAPK Tmk3 signaling in *Trichoderma atroviride.*** *Omar Nieves Ugalde*, Fidel Landeros Jaime, José Antonio Cervantes Chávez, Alfredo Herrera Estrella, Edgardo Ulises Esquivel Naranjo. Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro |
| **ST-50** | **Overexpression of nodulin 41 in transgenic roots of *Phaseolus vulgaris* and identification of its putative substrate.** *Juan Elías Olivares Grajales*, Marco Adán Juárez Verdayes, Chandrasekar Balu Rajeswari, Aarón Barraza Celis, Claudia Díaz Camino, Luis Cárdenas Torres y Federico Esteban Sánchez Rodríguez†. Instituto de Biotecnología, UNAM |
| **ST-51** | **Identification of proteins to the signal transduction in syncytiotrophoblast mitochondria.** *Sofia Nayeli Olvera Sánchez*, Óscar Flores Herrera, Mercedes Esparza Perusquía, Federico Martínez. Facultad de Medicina, UNAM |
| **ST-52** | **Activation of RhoGTPases, Rac and Cdc42, by calcium-sensing-receptor mutants found in breast cancer patients.** *Lennis Beatriz Orduña Castillo*, José Vázquez Prado, Guadalupe Reyes Cruz.CINVESTAV Zacatenco |
| **ST-53** | **Role of β-arrestin2 in signaling and regulation of Corticotropin-Releasing Factor Receptor Type 1 (CRF1).** *Gabriela Karina Parra Mercado*, Judith Hernández Aranda, Jesús Alberto Olivares Reyes. CINVESTAV Zacatenco |

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| **ST-54** | **The Human Papillomavirus type 16 E5 and Ras Oncoproteins control cell differentiation to favor transformation**. *Adolfo Pedroza Saavedra*, Giovani Visoso Carvajal, Lilia Chihu Amparan, Doris Arellano Quintanilla, Saraim Figueroa Brito, Minerva Maldonado Gama, Fernando Esquivel Guadarrama, Lourdes Gutierrez Xicotencatl. CISEI. Instituto Nacional de Salud Pública |
| **ST-55** | **TLR and MyD88 identification in the crayfish *Cherax quadricarinatus.*** *Mohamed Ali Pereyra Morales*, Oscar Vivanco Rojas, José Luis Sánchez Salgado, Juan José Alpuche Osorno, Ma. Guadalupe Maldonado Mercado, Concepción Agundis Mata. Departamento de Bioquímica, UNAM |
| **ST-56** | **Efecto del raloxifeno y el tranilast en la expresión proteica de e-cadherina en células de cáncer de mama.** *Jessica Trinidad Pérez Pérez,* Laura Cristina Berumen Segura, Guadalupe García Alcocer, Jesica Esther Escobar Cabrera. Unidad de Investigación Genética, Universidad Autónoma de Querétaro |
| **ST-57** | **Role of Ca2+ in the $a_1$-adrenergic receptor-mediated $H_2O_2$ synthesis.** *Enrique Piña Garza*, Raquel Guinzberg Perrsuquía, Antonio Díaz Cruzand, Magdalena Vilchis Landeros. Departamento de Bioquímica, UNAM |
| **ST-58** | **Paracrine Induction of Senescence by Mammalian Brain Cells *in vitro.*** Alexander Pommer Alba, Daniel Moreno Blas, Susana Castro Obregón. Instituto de Fisiología, UNAM |
| **ST-59** | **Dynamics of the phospholipid flippase DFN-2 *in Neurospora crassa.*** *Ivan Murillo*, Zachary Shultzhaus, Brian D. Shaw and Rosa Mouriño Pérez. Microbiología. Centro de Investigación Científica y de Educación Superior de Ensenada |
| **ST-60** | **TOR modulates AUXIN expression patters during root nodule symbiosis in common bean.** *Karen Ramìrez Méndez*, Manoj Kumar Arthikala, Miguel Lara, Kalpana Nanjareddy. Escuela Nacional de Estudios Superiores, Unidad León. UNAM |
| **ST-61** | **Dynamic network of Mdm2 and p53 interactions.** *David Ramírez Ramírez*, Vanesa Olivares Illana and Carlos Arturo Espinosa Soto. Instituto de Física, Universidad Autónoma de San Luis Potosí |
| **ST-62** | **Extracellular vesicles from women with breast cancer promotes migration in MDA-MB-231 breast cancer cells.** Javier Ramírez Ricardo, Octavio Galindo Hernandez, Pedro Cortes Reynosa, Eduardo Perez Salazar. CINVESTAV Zacatenco |
| **ST-63** | **Leptin regulates expression and subcellular localization of epithelial-mesenchymal transition markers in MCF10A human mammary epithelial cells.** Miriam Daniela Zuñiga Eulogio, José Luis Dena Beltrán, Mercedes Calixto Gálvez, Eduardo Castañeda Saucedo, Carlos Ortuño Pineda, Napoleón Navarro Tito. Laboratorio de Biología Celular del Cáncer, Universidad Autónoma de Guerrero |
| **ST-64** | **Participation of the MPK6-MKP1 module in the responses of the *Arabidopsis thaliana* root to L-glutamate.** *Gustavo Ravelo Ortega*, José López Bucio, Leon Ruiz Herrera, Arturo Guevara García y Jesús Salvador López Bucio. Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo |
| **ST-65** | **Role of UBEs AhR and RBX1 in the ubiquitin proteasome proteolytic pathway for Breast Cancer development.** Octavio Daniel Reyes Hernández, Claudia Vanessa Arellano Gutiérrez, Diana Casandra Rodríguez Ballesteros, Rosa María Navarrete Ramírez, Samantha Dayane Galván Reyes, Gisela García Casillas, Fabiola Fragoso Sandoval, Xicoténcatl Jiménez Villanueva, Carlos Ugarte Briones, Juan Carlos Bravata Alcántara, Gabriela Figueroa González, Sonia Chávez Ocaña, Mónica Sierra Martínez. Unidad de Investigación, Hospital Juárez de México |
| **ST-66** | **Biomodulators effect in the transformation of fibroblasts to myofibroblasts in an experimental model in vivo.** *Mónica Rivera Colin*, Gabriela Togno Latour, Alma Reyna Escalona Montano, María Magdalena Aguirre García, Augusto González Canto, Gudiño Zayas Marco Elias, Alfredo J. Gallegos Cigarroa, Ruy Pérez Tamayo. Facultad de Medicina. UNAM |
| **ST-67** | **Effect of fibronectin on acrosome reaction of guinea pig sperm.** *Ana Lilia Roa Espitia*, Irma Jiménez Morales, Humberto González Márquez, Enrique Othón Hernández González. CINVESTAV Zacatenco |
| **ST-68** | **Effect of fatty acids in adipogenesis.** *Damaris Rodríguez López*, Ma Cristina Amada Velez del Valle, Walid KuriHarcuch. CINVESTAV Zacatenco |

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| **ST-69** | **Apoptosis Analysis of a Lung Cancer Cell Line Resistant to Radiotherapy.** *Reynalda Roldán Pérez*, Elena Aréchaga Ocampo, Isidro Xavier Pérez Añorve, Claudia Haydée González de la Rosa, Rosalva Rangel Corona. Universidad Autónoma Metropolitana Cuajimalpa |
| **ST-70** | **MPK6 loss of function effects on the pattern of expression of the transcription factor ABI4 during embryo and root development and in response to ABA and glutamate in *Arabidopsis thaliana***. *Itzel Román Sánchez*, Ramos Vega Maricela, León Patricia, Guevara García Ángel Arturo. Instituto de Biotecnología, UNAM |
| **ST-71** | **Study of Ski transcriptional cofactor stability.** Reyna Edith Rosales Alvarez, Genaro Vázquez Victorio, Marina Macías Silva. Instituto de Fisiología Celular, UNAM |
| **ST-72** | **Isomers of conjugated linoleic acid induce insulin resistance through pkce activation in hepatic cells.** *Adriana Roura Guiberna*, Jesús Alberto Olivares Reyes. CINVESTAV Zacatenco |
| **ST-73** | **Interaction and regulation of retinoblastoma protein by Mdm2/Mdmx proteins in different conditions.** Adriana B. Rousset Roman, Jesús Hernández, Vanesa Olivares Illana. Instituto de Física, Universidad Autónoma de San Luis Potosí |
| **ST-74** | **Toll-like receptor 4 activation by lipopoly saccharide promotes progression in breast cáncer.** *Karina Ruiseco Flores*, Julieta López Cuevas, Eduardo Monjaraz Guzmán. Instituto de Fisiología, Benemérita Universidad Autónoma de Puebla |
| **ST-75** | **Effect of obesity on vascular reactivity and $Ca^{2+}$ homeostasis in *in situ* endothelial cells from female rat aorta.** *Josué Ernesto Sánchez Gómez*, Bricia Mani Zaca, Verónica Ajelet Vargaz Guadarrama, Roberto Berra Romani, Angélica Trujillo Hernández. Facultad de Medicina, Benemérita Universidad Autónoma de Puebla |
| **ST-76** | **Study of the regulation of the PI3-kinase/Akt pathway by the *O*-GlcNAcylation in cell lines derived from cervical cancer.** *Carlos Josué Solorzano Mata,* Edgar Zenteno Galindo, Cristina Castañeda Patlan, Yobana Pérez Cervera, Tarik Issad, Victoria Jiménez Castillo. Universidad Autónoma Benito Juárez de Oaxaca |
| **ST-77** | **Function of the Peroxisomal proteins Pex1, Pex4, Pex6 and Pex8 in the sexual development of the filamentous fungus *Podospora anserine*.** *Fernando Suaste Olmos*, Leonardo Peraza Reyes. Instituto de Fisiología Celular, UNAM |
| **ST-78** | **The actions of exogenous leucine on legume TARGET OF RAPAMYCIN signaling.** *Rosina Torres Ortega*, Kalpana Nanjareddy, Manoj Kumar Arthikala, Angeles Pérez Oseguera, Miguel Ángel Cevallos, Miguel Lara. Escuela Nacional de Estudios Superiores, Unidad León. UNAM |
| **ST-79** | **Participation of Actin Depolymerization Factor D (ADFD) in the early stages of the *Phaseolus vulgaris – Rhizobium* symbiosis.** *Roberto Toscano Morales*, Yolanda Ortega Ortega, Carmen Quinto Hernández. Instituto de Biotecnología, UNAM |
| **ST-80** | **Participation of STAT5 in the regulation of expression of GLUT-1 and hexokinase II.** *Arturo Valle Mendiola*, Isabel Soto Cruz, María del Carmen Lagunas Cruz, Benny Weiss Steider. FES Zaragoza, UNAM |
| **ST-81** | **Histamine Impairs Midbrain Dopamine Neuron Differentiation in Association to Modifications of Epigenetic DNA Marks.** *Fernanda Vargas Romero*, Ernesto Soto Reyes, Rodrigo González Barrios, Lissania Guerra Calderas, Iván Velasco. Instituto de Fisiología Celular Neurociencias, UNAM |
| **ST-82** | **Effect of Diabetes Mellitus Type 2 on the Regulation of Intracellular $Ca^{2+}$ in Excised Endothelial Cells from Rat Aorta.** *Verónica Ajelet Vargaz Guadarrama*, José Alonso Romero, Josué Ernesto Sánchez Gómez, Francesco Moccia, Franco Tanzi, Roberto Berra Romani. Facultad de Medicina, Benemérita Universidad Autónoma de Puebla |
| **ST-83** | **Effect of the adenosine derivative compound IFC-305 on activated Hepatic Stellate Cells.** *Gabriela Velasco Loyden*, Mariana Domínguez López, Jesús Rafael Rodríguez Aguilera, Julio Isael Pérez Carreón, Victoria Chagoya de Sánchez. Instituto de Fisiología Celular, UNAM |
| **ST-84** | **Expression of a specific peptide from SymHSP75, a light responsive protein from *Symbiodinium microadriaticum*, for polyclonal antibody raising.** *Marco A Villanueva Méndez*, Raúl Eduardo Castillo Medina, Tania T Islas Flores. Unidad Académica de Sistemas Arrecifales, Instituto de Ciencias del Mar y Limnología. UNAM |

# *Author Index*

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**N**



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Sociedad Mexicana de Bioquímica A.C.
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# Structural biology of TGF-beta family signaling proteins – new insights into mechanism to novel therapies for cancer and fibrosis

Andrew P. Hinck, Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA U.S.A.

Transforming growth factor-β (TGF-β) family signaling proteins, and many of their secreted antagonists, evolved from a common cystine knot growth factor (CKGF) fold in primitive metazoans. The TGF-β family has greatly diversified, with three family members in worms, seven in flies, and more than thirty in humans and other vertebrates. Through structural analysis, much has been learned about the molecular adaptations that the signaling proteins, single-pass transmembrane receptors, downstream effectors, and multitude of extracellular and intracellular modulators have acquired that enable the more than thirty proteins of family to achieve their unique biological functions [Hinck, et al, 2016]. In this talk, three recent examples will be presented which highlight how structural adaptations in the signaling proteins and receptors, as well as non-signaling co-receptors, engender the proteins of the family with their unique binding specificity and accordingly their unique functions. In the last part of this talk, two examples of protein-based inhibitors and one example of a small molecule-based inhibitor will be given as to how the unique modes of binding and adaptations can be exploited to develop novel therapeutic agents for treating diseases and disorders caused by aberrant pathway signaling.

Hinck, A. P., Mueller, T. D., and Springer, T. A., Structural Biology and Evolution of the TGF-beta family, Cold Spring Harbor Perspectives in Biology, *in press* (2016).

# Evolutionary implications of hybridization in fungi

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Hybridization creates opportunities for adaptive evolution and can cause immediate speciation, but also creates genomic challenges to overcome. Indeed, hybridization results in the combination of two diverged genomes, which are subsequently shaped by processes of recombination, deletion, and other genomic rearrangements. Genomics have recently paved the way to investigate the stochastic and adaptive processes that follow genomic hybridization. As compared to metazoans or plants, fungi have lower prezygotic barriers and can reproduce clonally for long periods of time, thus hybridization is thought to have a large impact in the evolution of this clade. Consistently, the presence of hybrids in fungi have been extensively documented and an increasing number of cases are being described in the literature. However we still lack a full understanding of how common and relevant has been this process in mediating the origin of new lineages and adaptation to new niches in fungi. Here, I will provide an overview of recent results from my group that showcase the study of ancient and recent hybridization events in fungi, and the follow up of the genomic aftermath of these hybridizations. Examples covered include the origin of the yeast whole genome duplication in Saccharomyces lineage, and the emergence of novel pathogenic lineages in the Candida parapsilosis clade.

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# DNA topology and the regulation of transcription

David Levens, Fedor Kouzine, Laura Baranello, Zuqin NIe, Damian Wojtowicz, Teresa Przytycka and Keji Zhao, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892

Intra- and extracellular signals are channeled through a multitude of specific and general transcription factors to RNA polymerase II (RNAPII) for regulate the synthesis of mRNAs, lnRNAs and other non-coding RNAs. The transcription machinery exerts mechanical force onto DNA and chromatin to expose the genetic information and copy it into RNA. During transcription the template DNA helix is overwound (positive supercoils) ahead of, and underwound (negative supercoils) behind the translocating RNAPII. This topological strain is not merely a by-product of transcription, but is a force that can be harnessed for regulatory purposes; negative supercoiling assists DNA melting that helps to assemble pre-initiation complexes (PICs), while positive supercoiling may help to destabilize nucleosomes. Supercoils also provide the free energy that supports the formation of alternative DNA structures such as single stranded loops, Z-DNA, quadruplex, and H-DNA that may serve regulatory functions. DNA is a tough material that resists bending and twisting, and so the over-accumulation of torsional stress may impede, pause or stall transcription unless properly disposed of. Topoisomerases (Tops) 1 and 2 handle the torque introduced by transcription or chromatin remodeling. Top1B by nicking one strand and allowing the free end of a broken strand to swivel about the unbroken strand, removes twist strain from a torqued segment of DNA. Top2, after introducing a double stranded break, passes an intact double stranded segment through the break prior to resealing it, and thereby reduces the writhing of DNA in 3-D to minimize topological strain. The removal of torsional stress by Top1 and Top2 is coordinated with, and managed by the transcription machinery. Top1 joins the PIC in an inactive state, but as the carboxyl-terminal domain (CTD) of the largest subunit of RNAPII is progressively phosphorylated by several kinases, including the kinase activity of the chromatin factor BRD4, the CTD is converted into an activator of Top1B. Other transcription factors have the capacity to activate Tops, and so management of DNA topology is likely to prove a key control point for high output gene expression. Intercepting the activation of Top1 and perhaps other Tops, during the transcription cycle may provide a therapeutic means to prevent the oncogenic high level transcription, such as the amplification of transcription in tumors driven by MYC.

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# Mitochondrial bioenergetics and OXPHOS supercomplexes.

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Mitochondria synthesize more than 90% of the ATP that cell needs to keep it away from thermodynamic equilibrium. For this, mitochondria use reducing coenzymes (*i.e.* NADH) or succinate to generate the proton electrochemical gradient ($\Delta\mu_{H+}$) across the inner membrane, and then use the energy stored into it to ATP synthesis. Energy transduction or oxidative phosphorylation is carried out by OXPHOS complexes (I, II, $III_2$, IV and V) which catalyze $H^+$ translocation to the cristae lumen (*i.e.* $\Delta\mu_{H+}$ generation) and its return to mitochondrial matrix (*i.e.* ATP synthesis). The arrangement and distribution of the OXPHOS complexes into inner membrane has been explained as a "plastic model"; in this model, respiratory complexes could be found as individual complexes or supercomplexes. On the one hand, self-association of complex V into a dimer ($V_2$), and these into oligomeric strings, has been related to cristae folding, particularly its border and tips. We have shown that regulation of dimerization of complex V is in collusion with cell differentiation and specialization, *i.e.* $V_2$ is present in human cytotrophoblast, a non-steroidogenic cell, while in syncytiotrophoblast, a steroidogenic cell, it is absent. Steroidogenic mitochondria are small and their architecture includes vacuolar cristae, which improve intramitochondrial cholesterol flux. Although $V_2$ structure and role in cristae folding are recognized, few studies have been performed about their bioenergetics role or kinetics. In this sense, $V_2$ isolated from *Ustilago maydis* mitochondria is six-times more active than $V_1$; but it´s three-time more sensitive to oligomicyn. Additionally, deletion of dimerizing subunit g in *U. maydis* didn't prevent complex V auto-association, but isolated $V_{2\Delta g}$ was inactive. Simultaneously, mitochondrial ATP synthesis from $\Delta$g strain was three-times lower that wt. This is the first report about the role of subunit g deletion in mitochondrial ATP synthesis. On the other hand, supercomplexes could contain complexes I:$III_2$, $III_2$:IV or I:$III_2$:IV (*i.e.* respirosomes). It has been proposed that their role involves substrates channeling or enzyme regulation. Isolated respirosomes from *U. maydis* show partial channeling and work as a single unit, without electron leaks during NADH oxidation and $H_2O$ production. A MS/MS analysis show the presence of 9 new proteins without a specific role described; particularly, two of them show transmembrane domains and glycine zippers; this structural characteristic could make of them the glue between complexes. Isolation of V2 and respirosomes could improve the knowledge about their function and regulation.

*Acknowledgments:* This wok was supported by Research Grant IN214914 (OFH), IN211715 (FM) and IN209614 (JPP) from Dirección General de Asuntos del Personal Académico (DGAPA) de la Universidad Nacional Autónoma de México, and 168025 (FM) from CONACyT. MEP is PhD student of Programa de Doctorado en Ciencias Biológicas de la UNAM.

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# Structural biology of organellar replisomes

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Mitochondria and chloroplast are organelles that contain their independent genomes. The evolutionary origin of their replication apparatus is mosaic (i.e. viral, bacterial and eukaryotic origins). We want to understand how organellar genomes are replicated and know the processes that regulated their faithful transmission to the next generation.

DNA replication is “bipolar concept”. In one hand evolution has generated mechanisms to assure the faithful replication of the genome, and in the other hand, without mutations evolution can not occur. In this talk we will present evidence that shows that organelar DNA polymerases are able to bypass DNA lesions with moderate fidelity. We postulate that DNA lesions may result in microhomology-mediated DNA replication by mitochondrial DNA polymerases. This aberrant replication process may account for the high-number of mitochondrial rearrangements. Thus, mitochondrial evolution is not due to the accumulation of point mutations, but by genomic rearrangements.

We postulate that priming at the organelar replication fork in yeast is mediated by a tripartite mechanism: an RNA polymerase, a specialized Primase-Polimerase and a primase-helicase. The interplay between these polymerases, will influence the number of mitochondrial rearrangements.

Finally, we will discuss the role of structural domains responsible in organellar DNA replication.

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# Roles of Non-Catalytic Residues in the Activity and Regulation of Plant ALDH10 Enzymes

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Plant ALDH10 enzymes catalyze the $NAD^+$-dependent oxidation of aminoaldehydes intermediates in polyamines catabolism or in the synthesis of the osmoprotectant glycine betaine (GB). Not all plants can synthesize GB in response to osmotic stress caused by drought, hypersaline soils or cold, and therefore engineering the synthesis of this osmoprotector in crops that naturally lack this ability has been a biotechnological goal for improving their tolerance to these very adverse environmental conditions. By crystallographic, kinetic, and site-directed mutagenesis studies we found that only those ALDH10s that have alanine or cysteine at position equivalent to 441 of the *Spinacia oleracea* enzyme (*So*BADH) efficiently oxidize betaine aldehyde (BAL) to GB, whereas those that have isoleucine cannot. The active site of the latter enzymes has a steric impediment that hinders the binding of the bulky trimethylammonium (TMA) group of BAL because isoleucine pushes Trp453—a residue that interacts with the TMA group trough cation-pi interactions—, thus narrowing the substrate-binding cavity. Phylogenetic analyses support the existence of two ALDH10 isoenzymes: the Ile441-type, which is present in most ALDH10 plants, and the Ala441- or Cys-441-type, which is present only in GB-accumulator plants.

By amino acid sequence analysis we found that a non-catalytic cysteine residue (Cys450, *So*BADH numbering) is strictly conserved in plant ALDH10s with proven or predicted betaine aldehyde dehydrogenase (BADH) activity, and highly conserved in those that oxidize other aminoaldehydes. Since no other enzyme of the extended ALDH superfamily has a cysteine at this position, we hypothesize that the non-catalytic Cys450 may play important role(s) in plant ALDH10s. Crystallographic evidence obtained with the *So*BADH enzyme indicated that Cys450 forms a thiohemiacetal with a BAL molecule, whose TMA group is in the same position as when BAL is productively bound. This accounts for the reversible and partial inactivation of *So*BADH by BAL in the absence of $NAD^+$. Accordingly, the C450S mutant enzyme was not inactivated, the A441C was inactivated similarly to the wild-type enzyme and the A441I—where the binding of the TMA group is hindered—required non-physiologically high BAL concentrations for inactivation. We propose that this novel covalent modification constitutes a regulatory mechanism of the activity of plant BADH enzymes. Under osmotic stress conditions, the partial and reversible inactivation by their physiological substrate BAL will prevent $NAD^+$ exhaustion while permitting the synthesis of high amounts of GB and avoiding the accumulation of the toxic BAL. Also, we have observed that the susceptibility to inactivation caused by oxidation by hydrogen peroxide of the C450S enzyme is significantly higher than that of the wild-type SoBADH. The protection against oxidation exerted by this residue might be the reason for its high conservation among plant ALDH10s, most of them peroxisomal or chloroplastic enzymes which are therefore prone to be subjected to oxidant conditions, particularly under osmotic stress.

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# tRNA loading by divergent pathways

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In most cases, two proteins that possess the same fold and function also share a common ancestor. They are called orthologs. For the majority of aminoacyl tRNA synthetases (aaRSs), ancient enzymes that attach amino acids to their corresponding tRNAs, this is almost always the case. However, glycyl tRNA synthase (GlyRS) comes in two forms. One form, a dimer, is present in some bacteria, archea and eukaryotes. The other, a heterotetramer, is found in most bacteria. Both forms share the same fold of the central catalytic domain (CCD). The relationship between these enzymes has remained a mistery. In this talk, we will describe the path that led us to define a new subclassification of aaRSs and to propose an evolutionary path of $\alpha_2\beta_2$ GlyRS, convergent with $\alpha_2$ GlyRS and divergent from AlaRS, thus defining the two types of GlyRS as isofunctional paralogs.

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# Protein characterization and biological model development from mass spectrometry data

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Most proteins are not a simple polypeptide exclusively defined by an amino acid sequence, but the result of multiple biochemical reactions. Many enzymes depend on a cofactor to be functional. Post-translational modifications play an important role in the regulation of cellular processes. Further, cleavage of the peptide chain might be involved in protein maturation, as well as degradation. Chemical derivatization of amino acids could be the result of natural protein aging or of reactions with xenobiotics. The biochemical state of a protein is related to its molecular weight. Therefore, the accurate determination of the protein mass, as well as the investigation of the amino acid sequence and the localization of chemical modifications by mass spectrometry play an important role in modern protein analysis. The talk will cover basics of protein mass spectrometry (MS) and present possibilities for top-down (whole proteins) and bottom-up (cleaved proteins, fragmentation studies) MS data analysis with freely available software. Additionally to the design of efficient data analysis workflows, data mining (DM) approaches will be introduced. DM models allow the development of predictive models, the identification of important variables and the detection of hidden associations. Thus, DM greatly supports the reliable classification of samples from MS data, e.g. in medical diagnostics, and the unbiased discovery of biologically relevant proteins and protein interactions.

# Biophysical approaches to studying the regulation of gene expression

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It's been more than 60 years since Watson and Crick solved the structure of DNA, using crystal diffraction data, and elucidated the central dogma of gene expression. Since then we have seen many extensions to the dogma as well as many other biophysical techniques used to study how genes are expressed and regulated in the cell.

I will present a short summary of my work to date, from using single molecule optical trapping techniques to characterize the mechanisms of bacterial transcription to conducting a systematic mapping of phosphosites in a protein involved in microRNA biogenesis. The emphasis of my talk will be on my latest project that focuses on resolving a longstanding controversy in the literature concerning the stoichiometry of Drosha, an RNaseIII enzyme, and DGCR8, a double-stranded RNA binding protein, within the Microprocessor Complex (MC). The MC is responsible for the first step in microRNA biogenesis. In collaboration with Dr. K. C. Neuman at the NIH/NHLBI, we devised a single-molecule subunit counting by photobleaching assay. I expressed full-length tagged constructs of both Drosha and DGCR8 in mammalian cells and isolated the MCs. Each protein was labeled with a different fluorophore. These MCs were then immobilized on a microscope coverslip and single molecules were imaged on a TIRF microscope. The number of photobleaching steps observed indcates that the MC is a heterotrimer composed of two copies of DGCR8 and one copy of Drosha.

# Cellular plasticity during neuronal differentiation and in the adult brain.

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Neurons derive from the progressive loss of plasticity of differentiating cells until get committed to a specific fate. The differentiation potential of neural precursor cells (NPCs) before commitment is defined by intrinsic properties but also depends on extrinsic factors such that markedly change when transplanted to ectopic places or cultured in vitro. Although commonly stem cells are the source of new cells in the adult organism, the ability to reprogram mammalian terminally differentiated cells into alternative fates, including returning to embryonic-like stages, prompts to consider more cellular sources for tissue repair than previously thought. In particular, neurogenesis in the adult brain has been considered restricted to specific niches where reside abundant neural stem cells (i.e., subventricular zone-olfactory bulb, hippocampus). However, neurogenesis in other brain areas has been controversial and cells giving rise to those putative new neurons have not been clearly identified. We recently found that the substantia nigra (SN), where the dopaminergic neurons that degenerate in Parkinson's Disease are located, allows efficient neuronal differentiation of transplanted primed embryonic stem (EB) cells, suggesting that the SN may contain a silent neurogenic niche. Accordingly, many host cells positive for the neuroblast marker doublecourtin (DCX+) emerge near the transplant. In the transplanted SN but not in the contralateral region, some host dividing neural NPCs Sox2+ are detected between 1-6 days post-transplatation (dpt) and possibly are the cells that give rise in the presence of Egf and Fgf2 *in vitro* to neurosphere-like aggregates with neural multipotency (i.e., differentiate into neurons and astrocytes). However, continuous BrdU administration after transplantation shows that only a fraction (~8%) of the host DCX+ progeny at 15 dpt derives from dividing cells and few BrdU+ cells survive up to 30 dpt. Interestingly, in addition of detecting an increased proportion of host GFAP+ expressing Nestin around the transplant (~50%), 30-50% of DCX+ or PSA-NCAM+ (also a neuroblast marker) cells express simultaneously the glial markers GFAP and S100β. In order to confirm that a large proportion of host putative neuroblasts originate from astrocytes, we have used a lineage tracing strategy in which EB cells are transplanted to brains of GFAP-CreER;R26-lox-mTomato-pA-lox-mEGFP-pA double transgenic mice that two weeks earlier are treated with tamoxifen for broad CreER activation in astrocytes. Consistent with an astrocytic origin of DCX+ cells, many cells surrounding the transplant are DCX+/EGFP+ and, later, many of these appear to become neurons (NeuN+). Preliminary data indicate that Fgf2 and VEGF are able to promote neurosphere formation and the emergence of DCX+ cells in the absence of EB cells. In addition, in contrast with other adult brain regions, we have been able to expand in vitro astrocyte-like cells from the SN. Together our data suggest that the adult SN has the ability to generate new neurons mainly from astrocytes by a dedifferentiation/transdifferentiation mechanism. We appreciate the technical assistance of M.Sc. Concepción Valencia and the financial support of CONACyT (grant 131031).

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# Synaptic and homeostatic plasticity orchestrating the persistence of memory

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One of the most important features of the nervous system is undoubtedly its ability to store information for long periods. The immediate question that arises is how the brain performs this function? Nowadays, cognitive neurosciences distinguish two types of plastic changes related to synaptic efficiency: the "Hebbian" and "homeostatic" plasticity. The Hebbian plasticity refers to a type of modification that tends to destabilize the properties of the neural network, causing a progressive change in the functions thereof. Such is the case of long-term potentiation (LTP). Meanwhile, the homeostatic plasticity refers to a type of modification which tends to stabilize the activity of the system modifying the response thresholds. Thus, Hebbian and homeostatic plasticity coexist, orchestrating the activity of neural networks. As part of our research we examined the interaction of training in an aversive behavioral task, conditioning taste aversion (CTA), which depends on the integrity of the insular cortex (IC), with subsequent expression of LTP, induced either by tetanic stimulation or by brain-derived neurotrophic factor (BDNF) administration in the IC. BDNF has recently emerged as one of the most potent molecular mediators of not only central synaptic plasticity, but also behavioral interactions between an organism and its environment. We also analyzed the cellular mechanisms involved in the actions of this neurotrophin on the retention of CTA. On the other hand, the hippocampal mossy fiber pathway (MF) constitutes a relevant area for the expression of structural and functional plasticity. Our studies show that application of high-frequency stimulation (HFS) sufficient to elicit LTP as well as acute intrahippocampal microinfusion of BDNF, induced synaptogenesis at the MF pathway. In addition, our results show that BDNF modifies the ability of this pathway to present subsequent LTP by HFS, and modifies the structural reorganization pattern. These findings support the idea that homeostatic forms of plasticity might provide the global regulation necessary to maintain synaptic strength and plasticity within a functional dynamic range.

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# Neurogenesis and sexual behavior

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Sexual behavior requires the expression and processing of olfactory cues by the main (MOB) and the accessory olfactory bulb (AOB). The olfactory bulb (OB) is one of the regions in the adult brain that receives and integrates new neurons that arrive from the subventricular zone of the lateral ventricles trough the rostral migratory stream. Previous studies from our group, demonstrated that the ability to control or pace the sexual interaction is crucial in male and female rats to develop a positive affective (reward) state. We have also shown that only when females pace the sexual interaction a higher number of new cells and neurons is observed in the AOB. When the females repeatedly mate pacing the sexual interaction a higher number of new cells is also observed in the MOB. The higher number of neurons is still present 45 days after the females control the sexual interaction suggesting that mating can induce permanent changes in the number of neurons that reach the AOB. The increase in the number of neurons after paced mating appears to be mediated by opioids because the injection of naloxone before mating blocks this effect.

Studies in male rats have also shown that only males allowed to control the rate of sexual interaction show an increase in the number of neurons in the AOB after mating. When males are allowed to ejaculate 1 or 3 times they show a significantly higher number of cells than males exposed to females. As well, males that mated with females that paced the sexual interaction showed a decrease in the number of BrdU+ cells compared with the control group and with those that mated freely. Together, these results indicate that the quality of stimulation that subjects receive when controlling the rate of the sexual interaction induces a higher number of neurons that reach the OB in rats.

Preliminary results in mice indicate, contrary to what we have observed in rats, that mating in males dose not induce a higher number of neurons in the OB after mating suggesting that the neurogenesis associated with mating could be species specific. We are also doing studies in the prairie vole to determine the contribution of neurogenesis induced by sexual behavior in a monogamous species.

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# Run for your neurons! Physical exercise and adult neurogenesis

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Abstract

The mammalian brain continuously generates new neurons in the dentate gyrus (DG) of the hippocampus throughout adult life. The integration of newborn neurons into the existing hippocampal neuronal network is considered physiologically important for learning and memory. A simple intervention like exercise, benefits learning and memory, and correlates with enhanced adult hippocampal neurogenesis and increased activity-dependent synaptic plasticity. To understand how exercise enhances memory function, it is essential to determine the modifications induced by physical exercise to the neuronal circuits of the newborn hippocampal neurons. Using a novel trans-synaptic tracing approach, we identified the monosynaptic inputs to the newborn hippocampal neurons in young adult male mice, under control or exercise conditions. One month of exercise increased neurogenesis in the dorsal, but not the ventral DG. Regional analysis of the traced cells indicated that exercise differentially affected specific inputs. Innervation from lateral entorhinal cortex was augmented and innervation from regions important for spatial memory and theta rhythm generation, including caudo-medial entorhinal cortex, medial septum and medial mammillary nuclei, were upregulated. Thus, exercise may facilitate learning and memory processes not only by a local increase in new hippocampal neurons, but also by the reorganization of new neuron circuitry.

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# Neurogenesis as a potential mechanism of repair after brain damage

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Brain damage leads to plastic events at the molecular and cellular levels. A salient response that occurs after damage is the increased proliferation of new neurons in the two well defined neurogenic niches, the subventricular zone and the dentate gyrus. However the role of adult-born neurons in a neurorepair process is still under debate. In order to address the potential contribution of adult-born neurons in morphological and functional reorganization, we have used an excitotoxic kainic acid-lesion model to induce focal damage to the dentate gyrus (DG). We have analyzed the naturally occurring morphological reorganization of the structure, but have also evaluated the impact of IGF-1 chronic infusion in the reorganization process. Along with the structural analysis, we have analyzed the maturation process of new-born cells surrounding the lesion, as well as the restoration of lost DG functions at a behavioral and electrophysiological levels. Lately, we have also addressed c-fos expression of neurons born after damage as well as of old neurons in face of a DG dependent task in order to correlate their activation with functional reorganization. Our main findings show that at 10 days after lesion the DG is disorganized and diminished in volume, rats do not recall an aversive context and DG LTP is abolished despite the significant increase in newly-born young and mature active neurons. At this time-point new neuroblasts expressing c-fos were found both in the granular layer and in the hilar region. At 30 days post lesion, the DG appears structurally reorganized and reaches the volumetric parameters of control subjects as shown through Cavallieri analysis. Such reorganization occurs along with LTP restoration and contextual fear recall. At this time point, the number of newly-born mature functional neurons is significantly higher as compared to the 10 days post-lesion group. Interestingly these cells were only observed in the granular layer suggesting that hilar new cells did not survive. Thus, the increase in new-mature neurons in the granular layer shows a time-dependent correlate with functional reorganization. In a different set of experiments, we observed that the chronic intracerebroventricular infusion of IGF-1 led to contextual fear memory recall as early as 12 days post-lesion together with the reduction of the lesion volume.

Our results show that the DG naturally reorganizes functionally and morphologically after a lesion but such processes can be accelerated in time after IGF-1 chronic infusion. Proliferation of new neurons is enhanced after a DG lesion and new neurons in the granular cell layer and hilus show to be active as early as 10 days post-lesion. At a later time point, newly-born mature active neurons outnumber those observed at 10 days post-lesion and are only observed within the granular cell layer, thus suggesting the possible contribution of such neurons in the reorganization process.

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# MAP kinase dynamics during cell-cell communication and fusion in *Neurospora crassa*

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Germinating conidia of many filamentous fungi sense each other and fuse. As a result, a germling network is formed, which further develops into the mycelial colony. Genetic analysis combined with live-cell imaging revealed an unusual mode of communication during these spore interactions in *Neurospora crassa*. The two fusion partners appear to switch between signal sending and receiving thereby establishing a kind of “cell-cell dialog”. This interaction involves the alternating recruitment of the MAK-2 MAP kinase module and the SO protein to the plasma membrane. Our further analysis revealed that the subcellular localization of MAK-2 strongly influences its activity. Tethering of the protein to the plasma membrane results in strong activation, probably by concentrating activators and/or the separation of inhibiting factors. In addition, we found that the composition of the plasma membrane is critical for a proper cell-cell interaction. Mutants accumulating specific ergosterol precursors are deficient in germling fusion, particularly in the processes after cell-cell contact. While the membrane recruitment of MAK-2 is mostly unaffected in these strains, SO strongly mislocalizes. SO interacts with another MAP kinase module, the MAK-1 pathway. In wild type, MAK-1 is recruited to the fusion point after cell-cell contact, but fails to accumulate in the sterol mutants. Inhibition of MAK-1 in a chemical genetics approach reproduces the phenotype of the sterol mutants. Together these data indicate that specific minor changes in the ergosterol molecule structure can exert major effects on specific signal transduction pathways.
In conclusion, our data suggest the presence of an intricate signaling network including two MAP kinase cascades controlling and linking cell-cell recognition, communication, and fusion.

# The MAPK signaling pathways: new roles in fungal physiology

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Cells respond to the environmental cues throughout different signaling pathways, since an appropriate perception is key for survival. Light is an abiotic factor that contribute as the main energy source on the earth and it also regulates the behavior in living forms. Recently, light response studies in different organisms, suggested that light could be a stress signal, which anticipate hazard conditions during the day. *Trichoderma atroviride* has three MAPK signaling pathways, which regulate development, cell wall integrity, and cellular stress. Specifically the kinases Tmk1 and Tmk3 regulate asexual spore formation triggered by injury and light. In yeast, there are evidences suggesting that the MAPKKK Ste11 regulates orthologues to Tmk1 and Tmk3, although in filamentous fungi Ste11 (MAPKKK) is specific for the activation of Tmk1 and the Ssk2 (MAPKKK) regulates the activation of Tmk3 orthologues. In *T. atroviride*, all evidence suggest that Ste11 regulates several stress responses through Tmk1 with similar or overlapping functions according to the results observed in *T. atroviride* mutants lacking Tmk3 and Pbs2; orthologous to yeast Hog pathway.

The MAPK Sty1 and SAPKA from *Schizosaccharomyces pombe* and *Aspergillus nidulans*, respectively, regulate stress response by the transcription factor Atf1. The phenotype of *T. atroviride atf*1 mutants was more severe than the phenotype displayed by Δ*tmk*3 and Δ*pbs*2 strains but apparently overlapping functions, suggesting that Atf1 is the target of Tmk3 but it still has independent roles on asexual reproduction and stress.

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# Phospholipid flippases and vesicle traffic during polarized growth.

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In fungal cells, specialized proteins gather in specific places to break cell symmetry and produce hyphae. This organization includes the orchestration of two distinct vesicle processes, endocytosis and exocytosis that take place in tandem in different areas of the apical compartment in growing hyphae. Part of the signals for endocytosis and endocytosis include the asymmetry of the plasma membrane phospholipid bilayer. We studied the flippases, DNF-1 and DRS-2 that seems to be responsible for this membrane asymmetry. The mutation of *dnf-1* and *drs-2* genes produced alteration in the maintenance and stability of the Spitzenkörper and affected the actin cytoskeleton organization in the apical compartment. Surprisingly, neither of the flippases DNF-1 and DRS-2 was present in the plasma membrane, both were localized in different layers of the Spitzenkörper, associated to different secretory vesicles. DRS-2 was associated to vesicles transporting chitin synthases. These results indicate that phospholipid flippases (P4 ATPases) may be important for polarity on secretory vesicles, Spitzenkörper integrity and thus for the localization of many tip growing proteins.

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# Insect endosymbionts: bacteria or organelles?

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In 1965, P. Buchner published his microscopic descriptions of intracellular "bacteria" found in a wide variety of insects. The study of these bacteria, known as endosymbionts, has advanced with the advent of genomics. In 2000 the first endosymbiont genome was published. It belonged to *Buchnera*, the primary endosymbiont of an aphid. Primary endosymbionts are indispensable for the survival of their hosts. *Buchnera* belongs to the γ-proteobacteria, but has a genome ten times smaller than that of *Escherichia coli*. The absence of various genes, including those of lipopolysaccharide biosynthesis, besides different auxotrophies, explain the inability of primary endosymbionts to grow in free-living conditions. Endosymbiont genome reduction, its transmission from one generation to another by maternal line and the supply of nutrients to its host has led to suggest that the endosymbionts are in the process of becoming organelles, besides illustrating the possible evolutionary path which led to the emergence of mitochondria. Recently the term "symbionelle" was proposed to designate the primary endosymbionts of insects.

Our work has focused on the study of native insect endosymbionts of Mexico, some with biotechnological interest such as the carmine cochineal, *Dactylopius coccus*, and a wax producing cochineal from Chiapas called niij, *Llaveia axin axin*. We found that both contain endosymbionts with small genomes. In carmine cochineals there are two species of *Wolbachia* (α-Proteobacteria very common in insects) and a new β-Proteobacteria that has genes for nitrogen fixation. This endosymbiont could compensate for low levels of nitrogen in the sap of the cactus from which the carmine cochineal feeds. The metagenome and metatranscriptome of the niij revealed that the primary endosymbiont was a Flavobacterium and its main role is to provide the essential amino acids that the host can not synthesize. We have also conducted studies of co-speciation between scale insects present in Mexico and their endosymbionts. The results showed that these insects commonly contained two endosymbionts, a Flavobacteria and an enterobacteria, which could indicate a metabolic complementation between these symbionts.

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# "Lessons on genomic plasticity from bacteria from microbial communities"

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My group has worked for many years with *Bacillus* spp. isolates from sediment from the Churince pond in Cuatrocienegas, Coahuila, as a model for understanding different aspects of community ecology. Our studies have uncovered an unsuspected inter- and intra-species diversity. In this talk, I'll give examples both of phenotypic and genotypic plasticity of some of the *Bacillus* spp.isolates that we have extensively studied. 'Reaction norms' obtained for different species revealed species differences in phenotypic plasticity, while genomic plasticity was explored through experimental evolution.

One common phenomenon in organismal biology is phenotypic plasticity: the capacity of a given genotype (or individual) to change its phenotype in response to a change in the environment. Phenotypic plasticity determines tolerance ranges of species when facing environmental challenges. Genotypic plasticity, on the other hand, implies genetic change. For bacteria, even on short timescales, evolution is one of the major ways in which they deal with drastic environmental variations. Many phenotypic traits are adaptations to environmental factors. The evolution of ecological niches concerns conditions under which species may evolve broad or narrow tolerance of abiotic conditions (i.e. temperature).

Regarding genotypic plasticity, we evaluated the capacity for the evolution of novel traits of six populations of *B. coahuilensis* strain (m2-6). Transposons' movement was a proxy for genomic plasticity, occurring after only 300 generations. The Biolog system was used toi evaluate adjustments in the evolved populations regarding carbon source utilization. The evolved strains, exhibited a general increase in the capability of using different sugars, with a parallelism of the different populations in the ability to use specific sugars and aromatic compounds. Similarly, all evolved populations showed a decrease in the use of some other sugars. We observed differences in the robustness of use of the different resources between the evolved populations. We suggest that the phenotypic optimization that occurs in any given medium that selects for optimal growth is probably the result of global changes in gene regulation in the evolved populations, that are therefore pleiotropic, and reflect the absence of a substrate-specific adaptation as a key driver of evolution.

Since the different *Bacillus* species isolated from the Churince pond have shared the same physical-chemical conditions for hundreds of years, we have a chance to ask whether they exhibit differences in their phenotypic plasticity to environmental parameters. Temperature appears as one of the factors of greatest effect on the ecology and adaptation of organisms. We found that the *subtilis*-species tolerate higher temperatures as compared to the *cereus*-species. The results showed that for temperature, reaction norms grouped the *Bacillus* species in a similar manner as taxonomic techniques. The phenotypic plasticities showed strong evidence of differential adaptive characteristics associated with evolutionary history.

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# Different Coats for Different Challenges: Adjusting the Bacterial Membrane to Distinct Environments

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Phospholipids are well-known for their membrane-forming properties and thereby delimit any cell from the exterior world. In addition, membrane phospholipids can act as precursors for signals and other biomolecules during their turnover. However, little is known about phospholipid function, and remodeling in bacteria.

*Sinorhizobium meliloti* is a Gram-negative soil bacterium able to establish nitrogen-fixing root nodules with their respective legume host plants, such as alfalfa. *S. meliloti* contains the negatively charged phosphatidylglycerol and cardiolipin as well as the zwitterionic phosphatidylethanolamine (PE) and phosphatidylcholine (PC) as major membrane phospholipids. In previous studies we had isolated *S. meliloti* mutants that lack PE or PC. Transcript profiles of mutants unable to form PE or PC are distinct; they differ from each other and they are different from the wild type profile. For example, a PC-deficient mutant of *S. meliloti,* which is unable to form nitrogen-fixing root nodules with host plants, shows an increase of transcripts that encode a possible lytic transglycosylase or enzymes required for succinoglycan biosynthesis and a decrease of transcripts that are required for flagellum formation. Indeed, a PC-deficient mutant is unable to swim and overproduces succinoglycan. Some suppressor mutants, that regain swimming and form normal levels of succinoglycan, are altered in the ExoS sensor and we show that the lack of PC in the sinorhizobial membrane is sensed by the ExoS/ChvI regulatory system. Therefore, the molecular mechanism for sensing the absence of PC from bacterial membranes seems to be indirect. PC-deficient membranes might be more permeable for protons leading to an untimely acidification of the periplasm, proteolytic degradation of the periplasmic ExoR repressor and premature signaling through the ExoS/ChvI two-component system. The malfunction of the ExoS/ChvI system in PC-deficient *S. meliloti* mutants might explain their inability to establish a symbiosis.

Bacterial membrane lipid composition should not be considered as an invariable constant, but rather as the result of a steady-state, characteristic for a given physiological condition. Under certain stress conditions, specific new membrane lipids can be formed in order to minimize the stress exerted. For example, under phosphorus-limiting conditions of growth, bacteria such as *S. meliloti* form membrane lipids lacking phosphorus such as ornithine-containing lipids, or the diacylglycerol (DAG)-based sulfolipids, and betaine lipids. Also, bacteria entering stationary phase of growth liberate vast amounts of free fatty acids. Mechanistic details of these lipid remodeling processes will be shown.

# Evolution of mitochondria: when, where and from which bacteria?

**Mauro Degli Esposti**
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This paper will discuss recent developments on the origin of mitochondria and related organelles of eukaryotic cells from bacterial ancestors. The topic is becoming more and more hot after the recent discovery of a potential close relative to the archaean host that might have engulfed, or be parasitized by the bacterial guest that then became the mitochondrion of our cells. The topic generates controversy regarding, in particular, when did this bacterial guest associate with the proto-eukaryotic cell. Evidence will be presented that re-enforces earlier proposals that a single alpha-proteobacterium could have been the progenitor of both the aerobic mitochondrial metabolism and also of the anaerobic metabolism that is displayed by extant eukaryotic organisms adapted to anaerobic conditions such as *Entamoeba*.

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# Are essential genes always part of the core genome?

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Bacterial genomes are mosaics, in the sense that different individuals belonging to the same “species” (we use the term ‘species’ here in the operational way discussed below), share only a limited part of their genomes, while other parts are only present in a limited number of individuals, or are unique to only one member of the species.

This high genomic variability has important implications for bacterial taxonomy, both practical and at the theoretical level. Thus, it is not feasible to establish a congruent definition of a bacterial species as a reproductive isolated group of individuals that have a common evolutionary history. The operational definition of a bacterial species is the group of clones or individuals that have unique phenotypic properties, have more than 98.7% sequence identity of the 16S rRNA gene and exhibit more than 70% DNA hybridization.

In order to do phylogenetic studies of bacteria, their genomic information has been classified in three major classes. The core genome has been defined as the part of the genetic information that is present in all individuals of the same “species”. The accessory genome (which has also been considered as the “adaptive” genome) is present only in a part of the population or in one individual and is acquired by horizontal gene transfer (HGT) The sum of the core genome and the accessory genome is the pan-genome, which represents all the genetic repertoire of a bacterial species.

Even though the term core genome is defined as an intra-species concept, it is deduced that, being the information that defines the basic functions of the biology of a species, it should have as a coherent ensemble with a relation of ancestry with the core genome of other bacterial species. This is the rationale for constructing phylogenetic trees with ribosomal and other universal genes to obtain a bacterial taxonomy.

It is common to assume that the core genome contains all the genes that essential for the biology of a determined bacterial “species”, but essential genes for the biology of a group of bacteria refers to the function of the genes, while the concept of core genome is a concept based on the conservation of DNA sequences. It is then plausible, that essential genes are not part of the core genome, if two unrelated DNA sequences, inherited by HGT, encode interchangeable functions.

In order to find examples of essential genes that are not part of the core genome, we have analyzed the annotated genomes of 63 *Escherichia coli* strains. The results will be discussed in the light of models to explain bacterial evolution.

# An autophagy-related kinase is essential for *Phaseolus vulgaris* symbiosis with both *Rhizobium* and arbuscular mycorrhizal fungi

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[¶] In loving memory

Eukaryotes contain three types of lipid kinases belonging to the phosphatidylinositol 3-kinase (PI3K) family. In plants and *Saccharomyces*, only PI3K class III family members have been found. These enzymes regulate the innate immune response, intracellular trafficking, autophagy and senescence. Here, we report that RNAi-mediated down-regulation of *P. vulgaris PvPI3K*, severely impaired symbiosis in composite common bean plants with endosymbionts such as *Rhizobium tropici* and *Rhizophagus irregularis*. When *PvPI3K* was downregulated, a marked decrease in root hair growth and curling was observed. Additionally, infection thread growth, root-nodule number, and symbiosome formation in root nodule cells were also severely affected. Interestingly, root colonization by AM fungi, the formation of arbuscules was also abrogated in PI3K loss-of-function plants. Furthermore, the transcript accumulation of genes encoding proteins known to interact with PI3K to form protein complexes involved in autophagy was drastically reduced in these transgenic roots. Expression of one of these genes, *Beclin1* / *Atg6,* was also knocked-down by RNAi, resulting in a similar impaired-symbiosis phenotype as that observed in *PvPI3K* down-regulated transgenic roots. Our findings show that an autophagy-related process is crucial for the mutualistic interactions of *P. vulgaris* with beneficial microorganisms.

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# Origin and genome shaping of common bean, from uncovering its closest sister species to its domestication in America

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Modern civilization depends on only a few of the estimated world's 300,000 plant species for its nourishment, including common bean (*Phaseolus vulgaris*), the most important grain legume. These crop plants were domesticated and improved by thousands of years of human selection, which transformed wild ancestors into high-yielding domesticated descendants. Therefore, defining the loci and associated polymorphisms behind the emergence of domestication and improvement traits in *P. vulgaris* is of major importance.

By integrating genomic, phylogenomic and metabolomic data from thirty sequenced accessions representing 12 *Phaseolus* species, we reconstructed an evolutionary model of common bean lineage divergence in the Americas that sustains the Mesoamerican origin of *P. vulgaris*. Our results demonstrate a speciation event in the Peruvian-Ecuadorian region of the tropical Andes that precedes the split of the Mesoamerican and Andean gene pools, defining the closest sister lineage of *P. vulgaris*, which appears to be preferentially autogamous.

We further uncovered intra- and inter-species introgression events that indicate transfer of stress response genes, which together with domestication protein coding and non-coding genes gave rise to domestication and adaptive traits.

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# Multidisciplinary Research Needed for Sustainability of Global Phosphorus

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Phosphorus (P) is one of 17 essential elements required for plant growth. Moreover, the human body contains some 700g of P derived either directly or indirectly from plants. While most soils contain P much of it is bound and unavailable for plant growth and development. This necessitates application of P fertilizers to soil to maximize high quality food and fiber from plants. Recent projections suggest that by 2050 the amount of P fertilizer needed to feed the world's 9 billion people will be 40 Tg/year, doubling current usage. Most P fertilizer originates from mined rock-P, a non- renewable resource. World rock-P reserves are not equally distributed geographically. Thus, the use of P fertilizer for agriculture poses issues related to sustainability and health. In the developed world overuse of P fertilizer has contributed to eutrophication of large bodies of water. While in the developing world inadequate availability and the high price of fertilizer P contributes to lower crop yields and poorer quality. Within the last 15 years plant biologists have unraveled biochemical and molecular mechanisms that contribute to plant adaptation to low soil P, identified genes involved in P acquisition and use, and made progress in finding genotype with improved growth on low P. Microbiologists have identified soil microbes that can release P bound to soil particles. Moreover, economist and environmental scientists have detailed how humans have impacted earth's geochemical P balance. The future sustainability of P will require multidisciplinary collaboration to improve crop use of P fertilizer, find methods to establish P-solubilizing microbes to increase availability of soil-bound P, develop simple measures to recycle P from human and animal waste and ameliorate eutrophication of water.

This presentation is dedicated to honor the career and memory of Professor Dr. Federico Sanchez.

# Orchid diversity in Mexico

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The orchid family (Orchidaceae, Asparagales, Monocotyledoneae) is arguably the largest group of flowering plants, with ca. 30,000 extant species. Most such diversity is concentrated in the tropical regions of the world, with the Neotropics (the tropical portions of North- and South America and the Caribbean) hosting nearly one-half of their global diversity. Mexico represents the northern limit of the Netropics, and their nearly 2 million square kilometers sustain a diverse orchid flora, which includes four of the five main orchid lineages (formally recognized as subfamilies), represented by 14 tribes, 23 subtribes, 166 genera, and 1,266 species. The phylogenetic relationships of the Mexican orchids at the subfamilial, tribal, and subtribal levels are firmly established as a result of recent analyses of DNA sequences from multiple plastid and nuclear genes and non-coding regions. However, interspecific relationships and generic limits require further study. A quantitative analysis of the distribution of orchid diversity in the biomes and biogeographic units of this country demonstrates that such diversity is very unevenly distributed, with a disproportionately high proportion (70% of the species) concentrated in the mountain cloud forest, which cover less than 2 % of the territory. Exploration of previously inaccessible areas and careful systematic revision of little-studied groups continue to reveal new species and new records, and our estimates indicate that only about 80% of the orchid diversity expected for this country has been documented so far.

# Gpn1 and Gpn3, a shared adventure.

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Among the GTPase superfamily there is a group of GTPases called GPN-loop GTPases that received their name from the presence of a conserved GPN (glycine-proline-asparagine) motif. The group includes three members, Gpn1, Gpn2 and Gpn3, all derived from a single ancestral gene in Archaea. These proteins are universally expressed in eukaryotic cells and are essential in all tested biological models. The essentiality of these GTPases is probably due to the fact that they are somehow involved in nuclear targeting of RNA polymerase II, the enzyme that transcribe, among other, all protein coding genes. Interestingly, it has been reported that Gpn1 and Gpn3 are both involved in the same cellular functions. We will talk about our efforts at the Physics Institute of the Autonomous University of San Luis Potosi to define the cellular functions of Gpn1 and Gpn3, as well as the underlying molecular mechanisms. One strategy consists in replacing endogenous Gpn1 or Gpn3 by recombinant tagged versions of these proteins, which allows us to easily purify them or to follow their subcellular distribution in human cells. To this end we employ two retroviral vectors, one to express wild type or mutant versions of Gpn1 or Gpn3, and a second one to produce an shRNA highly effective to suppress the expression of the corresponding endogenous protein. Importantly, the cDNA to express the recombinant versions of Gpn1 or Gpn3 contains a mutation that makes it resistant to this shRNA. With this approach we investigate possible alterations in the cellular functions of Gpn1 or Gpn3 caused by mutations described in human tumors. This strategy also allows us to study the functional importance of specific residues in putatively important motifs in Gpn1 or Gpn3. Part of our effort is focused on the purification of recombinant Gpn1 and Gpn3 produced in bacteria with the goal of performing *in vitro* assays to define the biochemical and biophysical properties of these essential GTPases.

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# Regulation of the innate immune response of the intestinal epithelial cells by PI3K/Akt signaling during colitis.

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Epithelial cells lining the intestinal tract form a selective barrier that regulates nutrient uptake and limits exposure to luminal antigens and toxins. Thus the intestinal epithelium is consider as part of the nonspecific defense mechanisms that come into play after antigen exposure which is referred as the innate immunity system. The intestinal epithelium is highly dynamic and actively turned over. Therefore the intestinal homeostasis is maintained by tightly regulated mechanisms including stem cell proliferation, differentiation, migration and apoptosis. Accumulating evidence suggests that the barrier properties of the intestinal epithelium, which are important part of the innate immunity system, are compromised in Inflammatory Bowel Diseases (IBD). In fact in animal models, increased paracellular permeability across intestinal epithelial cells has been observed prior to the onset of inflammation. Increased paracellular permeability enhances antigenic exposure to underlying immune cells thereby further compromising epithelial barrier function and creating a vicious cycle that leads to development of chronic inflammation. Altered intercellular junction function, micro-erosions and increased epithelial apoptosis are the main contributors to epithelial barrier breakdown observed during colitis. For that reason, impaired homeostasis and destruction of cell adhesion centers are important pathophysiological events in the development of IBD. Intercellular junctions encompassing Tight Junctions (TJs), Adherens Junctions (AJs), and Desmosomes (DMs) play a pivotal role in regulating epithelial barrier properties. The studies in my lab highlighted the importance of DMs and Akt signaling in regulating intestinal epithelial barrier function and their contribution to the pathophysiology of mucosal inflammation and currently we intend to define the mechanisms by which inflammation regulates intestinal epithelial homeostasis (proliferation and apoptosis) and epithelial barrier function through inhibiting β-catenin signaling. Our current studies demonstrate that PI3K/Akt signaling plays an important role in the inhibition of □-catenin co-transcriptional activity and we have evidenced that this process plays an important role in the destruction of the intestinal epithelial barrier. We strongly believe that identifying new molecules that regulate the functions of the innate immune system (epithelial barrier properties) will help in defining new therapeutic strategies aimed to ameliorate the symptoms experienced by IBD patients.

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# Functional interaction of hypoxia-inducible factors and autophagy mediates drug resistance in colon cancer cells.

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Resistance to chemotherapy is the primary cause for treatment failure in clinical oncology. Hypoxia and accumulation of hypoxia-inducible factors (HIFs) in solid tumors have been associated with resistance to chemotherapy and poor prognosis. HIFs also causes autophagy establishment, particularly in RAS-driven and B-RAF-driven cancers, such as most colorectal carcinomas. However, the relevance of the relation between HIFs and autophagy in drug resistance is not well understood.

In this study we examined the effects of stable knockdown of HIF-1α or HIF-2α expression on autophagy and drug resistance displayed by colon carcinoma cell lines. We found that malignant cells exhibit high basal levels of autophagy and co-expression of HIF-1α and HIF-2α, compared with control non-malignant cells. HIF-1α or HIF-2α depletion alone resulted in increased autophagic and apoptotic cell death, indicating that survival of colon cancer cells is HIF-dependent. Autophagy inhibition with hydroxychloroquine (HCQ), despite induced additional increase in autophagy, did not sensitize SW480 cancer cells to the mTOR inhibitor temsirolimus (CCI-779) nor to 5-fluorouracil treatment, compared with control untreated cells. Strikingly, we found instead, that the most effective way to induce massive cell death via apoptosis and to avoid resistance to drug treatment, was the combination of drugs, particularly CCI-779, with the silencing of HIF-2α, or even with the only silencing of HIF-2α, emphasizing the crucial role played by HIF-2α in the promotion of cell survival and resistance to death.

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# *EMT and migration in ovarian carcinoma cells are regulated by UTP and adenosine.*

## Señalización por nucleótidos y nucleósidos en células derivadas de carcinoma ovárico: un mecanismo autócrino-paŕacrino que modula la migración y la invasividad celular .

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Nucleotides and nucleosides are signaling molecules that have a variety of roles mediating paracrine or autocrine activities by acting through specific membrane receptors. Their participation in cancer has been studied but it is still not clear. In this work, we studied the role played by UTP and adenosine in the migration of ovarian carcinoma-derived cells SKOV-3.
Stimulation of carcinoma-derived SKOV-3 cells with UTP (100 µM) increased migration (≈57%), while apyrase (10 U/mL), an ectonucleotidase that catalyzes dephosphorylation of purine and pirimidine nucleotides, decreased basal migration (≈47%). P2RY2 was found to be the receptor mediating these effects because the knock down of this receptor blocked the UTP-induced cell migration, and was dependent on EGFR transactivation. UTP effect on migration was also associated with epithelial to mesenchymal transition (EMT), since it was associated with an increase of *snail* and *twist* expression, known EMT inductors, as well as an increase of vimentin expression, a marker protein for mesenchymal phenotype.
In turn, the inhibitory effect of apyrase over SKOV-3 basal migration was associated with an enrichment of E-cadherin in the cell contacts, suggesting the establishment of an epithelial phenotype. This observation strongly suggests the possible role of adenosine inhibiting the invasive ability in these cells.
To analyze the effect of extracellular adenosine over cell migration, we studied the effect of adenosine and a set of drugs that modify the adenosine activity, adenosine 5'-(α,β-methylene) diphosphate (APCP), an inhibitor of the enzyme that turns AMP into adenosine (NT5E); adenosine deaminase (ADA), the enzyme that catalyzes the desamination of adenosine to inosine; dipyridamole (DPR), an inhibitor of the adenosine uptake mediated by concentrative nucleoside transporters (CNT). By blocking NT5E enzyme with APCP and degrading adenosine with ADA, migration was unaltered even in the presence of apyrase. However incubation with DPR induced a reduction of basal migration (≈36%), that was even more accentuated with adenosine (100 µM) (≈64%), suggesting that extracellular adenosine could be acting upon ADORA receptors.
Our results suggest that released nucleotides acting upon P2RY2 receptors are inductors of mesenchymal phenotype, while adenosine acting over an ADORA receptor could have antagonistic effects by promoting an epithelial phenotype in ovarian carcinoma cells.
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# G-proteins regulate mitotic spindle formation

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# Positive effects of (-)-epicatechin and epicatechin rich cocoa on indicators of mitochondrial biogenesis, oxidative stress in senile mice and in patients with type 2 diabetes.

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Aging and Type 2 diabetes (T2D) are associated with high levels of skeletal muscle (SkM) oxidative stress (OS). Aging presents a progressive decline in the functional maintenance of tissue homeostasis accompanied by an increase propensity to develop degenerative conditions that when combined with pathologies like T2D increase cardiovascular risk. It has been proposed that an increase in reactive oxygen species (ROS) generation leads to OS contributing to the aging process and to the worsening of T2D. Dysfunctional mitochondria are considered as a main source of ROS within the cell, their healthy renewal underpinned by mitochondrial biogenesis process may be impaired with aging and T2D.

Health benefits attributed to flavonoids have been ascribed to antioxidation. However, for flavonoids with similar antioxidant potential, end-biological effects vary widely suggesting other mechanistic venues for reducing OS. Decreases in OS may follow the modulation of key regulatory pathways including endogenous antioxidant levels and enzymes such as mitochondrial superoxide dismutase and catalase.

We evaluated the capacity of (-)-Epicatechin (Epi), a naturally occurring flavonoid, to reduce aging-induced OS and restore mitochondrial biogenesis as well as structural and functional indicators in aged mice and the effects of Epi-rich chocolate in SkM mitochondrial structure and mediators of biogenesis, in T2D patients.

Epi was able to restore the antioxidant defenses system and mitochondria markers in senile mice, Epi-rich cocoa treatment improves SkM mitochondrial structure and increases molecular markers of mitochondrial biogenesis resulting in enhanced cristae density. Altogether, these data suggest that Epi is capable of modulate OS.

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# Visualization of reactive oxygen species dynamics in developing zebrafish embryos.

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Reactive oxygen species (ROS) are natural oxygen derivatives generated during aerobic metabolism and by specific enzymatic activities. ROS play pivotal roles in the regulation of major cellular behaviors such as proliferation, cell death, cell migration and cell differentiation, all fundamental during animal development. Previously, we reported that ROS participate in the control of cell death during morphogenetic remodeling of different developing tissues in mouse embryos, suggesting an extensive role in development. To gain further insight into the role of ROS during development, zebrafish embryos were stained with a widely used ROS-sensitive dye and visualized by real time 3D confocal microscopy. Interestingly we found that ROS present highly dynamic patterns that correlate with key developmental process. For instance during early cleavages, ROS was observed at the cleavage furrows. In later 32-cell to 64-cell stage embryos, ROS staining was still present at cleavage furrows, but the signal presented intensity fluctuations seemingly coordinated to cell divisions that later on show an apparent “wave” pattern. Remarkably we found that during gastrulation and in particular in epiboly, that is the first major morphogenetic event in which massive movement of cells occur, ROS exhibited dynamic fluctuations among cells, in addition to a distinctive intense region at the margin of moving cells during whole epiboly progression. We found that the observed ROS are generated by NADPH oxidase (Nox) activity and that specific pharmacological inhibition of Nox, decreased cell motility and delayed epiboly, an affect that can be fully rescued by hydrogen peroxide treatment. Therefore in the present study we show evidence that ROS participate in the control of gastrulation a major developmental process that is fundamental in animal development. **Supported by PAPIIT/UNAM IN205612 and IN210316.**

**The several faces of the P2X7 receptor: a cation cannel that triggers reactive oxygen species production, cell death and interleukin production.**

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Trimeric P2X receptors (P2XR) are cation channels gated by extracellular ATP (ATPe). These receptors play a fundamental role in heart, kidney, the gastro-intestinal system, the immune system, epithelia, and the central nervous system. Seven P2XR (P2X1-P2X7) have been characterized. Some receptors form hetero-trimers. However, P2X7R only form homo-trimers and were thought to work alone. Recently, we demonstrated that P2X7R function is influenced by the presence of P2X4R. Such interaction between P2X7 and P2X4 does not involve channel activation. The interaction occurs when P2X7, but not P2X4R, is activated. We have shown that both receptors are near each other in the plasma membrane since they co-immunoprecipitate. Furthermore, once P2X7 is activated by ATPe both proteins come in close proximity and produce a FRET signal. Such dynamic interaction is due to the presence of the large carboxyl tail of P2X7 since removal of this protein segment ablates the interaction. Finally, the physiological relevance of such interaction was evaluated using $P2X4^{-/-}$ mice. Peritoneal macrophages that express both receptors were isolated and used to evaluate cell death, interleukin-1beta release, and uptake of large dye molecules. These functions are all triggered by P2X7R but we found that in $P2X4^{-/-}$ macrophages all functions were severely handicapped. Thus we proposed that the regulation of P2X7R by P2X4 is physiologically relevant and it is attributable to a protein-protein interaction.

Since phagocytic macrophages require reactive oxygen species (ROS) to kill pathogens, we characterized the signalling cascade leading to ROS production after activation of P2X7R by ATPe. Our data show that ROS production was dependent on intracellular calcium entry via P2X7R. Stimulation of P2X7R led to activation of Pyk2 but not calmodulin. Additionally, inhibitors of MEK1/2 and c-Src abolished ERK1/2 activation and ROS production but inhibitors of PI3K and p38 MAPK had no effect on ROS generation. Activation of ERK1/2 was also abolished by PKC inhibitors, but the inhibitors barely reduced the amount of ROS produced by Bz-ATP. In agreement with this, the amount of ROS produced by PMA was about half of that produced by Bz-ATP. Taken together our results suggest that activation of P2X7R leads to activation of the PKC/c-Src/Pyk2/ERK1/2 pathway and the subsequent production of ROS by the NOX2 enzyme, a target of the kinase ERK1/2.

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# REACTIVE OXYGEN SPECIES AS KEY REGULATORS OF POLAR GROWTH AND SYMBIOSIS

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In plant cells ROS accumulation have been involved in several processes such as: development, hypersensitive response, hormonal perception, gravitropism and stress response. In guard cells from *Vicia faba* regulates the opening of stomata and more recently in root hair cells from *Arabidopsis* ROS levels generate and maintain an apical calcium gradient. This ROS accumulation plays a key role in root hair tip growth and suggested to play a similar role in pollen tubes and other tip growing cells. Furthermore, the enzymes generating ROS such as the NADPH oxidases, also plays a key role during the pathogenic and mutualistic interactions.

Herein we report a new molecular probe to depict the ROS dynamic during root hair cell and pollen tube apical growth. Hyper is a new generated GFP fused to the OxyR domain that result in a hydrogen peroxide specific probe. This molecular probe was expressed in root hair cells from Arabidopsis and tobacco pollen tubes (1). By using high resolution microscopy we depicted an apical $H_2O_2$ gradient at the tip dome where the polar growth occur, furthermore we were able to visualize dynamic ROS oscillations in root hair cells, which are couple to growth. In pollen tubes we also found a particular ROS distribution, with clear oscillations couple to growth fluctuations. In both tip growing cells, the apical regions are the site where the more dynamic ROS changes were observed, suggesting a pivotal role in polar growth. The implication for the rhizobia-legume interaction will be also discussed.

1. Hernandez-Barrera, A., Quinto, C., Johnson, E. A., Wu, H. M., Cheung, A. Y., & Cardenas, L. (2013) *Methods Enzymol* **527,** 275-290.

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# Peroxisome and mitochondrial dynamics during sexual development of the fungus *Podospora anserina*

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Mitochondria and peroxisomes are dynamic organelles that coordinately mediate fundamental cellular processes essential to development. These two organelles have a close relationship that includes cooperative roles in the metabolism of reactive oxygen species, lipids and carbon intermediates used as source of energy and biosynthetic metabolites. Peroxisomes and mitochondria importantly contribute to redox homeostasis and signaling. Furthermore, the dynamics of these organelles is controlled by common proteins, and they maintain a redox communication that modulates their dynamics and activity. In the fungus *Podospora anserina*, different peroxisomal and mitochondrial functions are required at specific stages of sexual development. We have shown that the protein machinery mediating peroxisome biogenesis is regulated during sexual development, and that absence of distinct peroxisome biogenesis factors differentially affects both sexual development and mitochondrial dynamics. Here we show that peroxisomes adapt their dynamics in response to metabolic and environmental cues, including the redox state of the cell. In addition, we have discovered that peroxisome dynamics is also very highly regulated during sexual development, in close correlation with the functional state of the proteins controlling peroxisome assembly. Moreover, we demonstrate that the dynamics of both peroxisomes and mitochondria depend on the protein FIS1, and we have analyzed the effect of eliminating this fission factor in sexual development. Our findings are consistent with a high regulation of peroxisome and mitochondrial dynamics during the fungal life cycle. This research was supported by PAPIIT grant IA201815 from DGAPA-Universidad Nacional Autónoma de México.

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# Mitochondrial proteomic analysis during development of chemically induced hepatocellular carcinoma in rats: an evolutionary perspective

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Otto Warburg put forward the hypothesis the cancer cells arise from normal cells in which the mitochondrial respiration has been damaged. During several selective cell divisions, these cells increase their capacity for anaerobic fermentation (glycolysis to produce lactic acid), to compensate energetically their initial loss of respiration. Thus, cancer development can be seen as a microevolutionary process in which the cells are positively selected for their ability to proliferate, and this ability is related to metabolic changes in which mitochondria have a preeminent role. On this basis, we postulate, as a working hypothesis, that the changes observed in the nuclear-mitochondrial interactions during cancer development, recapitulate the co-evolutionary process that forges this interaction. We are using the hepatocyte resistant model in rat to follow changes in nuclear-mitochondrial interactions during cancer development. In this model, hepatocellular carcinoma (HCC) is chemically induced in a controlled and precise manner. We conducted a massive comparative mitochondrial proteomic analysis by tandem MS/MS coupled to liquid chromatography to study HCC development from early to advanced stages. Our analysis suggest that the expression of proteins involved in carbon metabolism and pyruvate import to mitochondria is decreased since early stages after the HCC induction. In persistent nodular tissues (at 9 months), we detect differentially expression of proteins involved in carbon sources characteristics of cancerous cells, as glutamine. In tumoral tissues, we observed an increment in expression of proteins from several pathways, the most affected involved protein processing and amino acids metabolism. A decrease in the expression of proteins of the cytochrome c oxidase complex was found, and we concordantly found that its oxidation activity was also diminished. To put our results in evolutionary context, we compare them with preliminary genome-wide scans of positive selection.

# Anti-mitochondrial therapy against malignant tumors

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The metabolic reprogramming in tumor cells is related to an elevated glycolytic rate mediated by the activation of some transcriptional factors (HIF-1α) and oncogenes (c-MYC, h-RAS). In malignant tumors, the glycolysis activation has been frequently associated with an impaired mitochondrial function. However, it has been lately demonstrated that metastasic cancer cell lines grown as monolayers or microspheroids show a functional oxidative phosphorylation (OxPhos) which provides more that 75% of the ATP required for growth and other cell functions. As a higher electronegative mitochondrial membrane potential has been observed in cancer cells vs. non-cancer cells, it seems plausible to target cancer mitochondria with lipophilic cations such as casiopeina II-gly, rhodamines and vitamin E-phosphonium derivatives. Indeed, these lipophilic cations at submicromolar, therapeutic doses inhibited cancer cell growth, decreased mitochondrial protein contents and abolished OxPhos enzyme activities and flux, with no apparent effect on non-cancer cells. Therefore, anti-mitochondrial therapy emerges as a potential alternative to deter malignant tumor growth.

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# Systematic identification of cellular-aging factors and dietary-restriction effects

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Dietary restriction --the limitation of calories or specific nutrients in the diet-- is the only non-genetic intervention known to increase the longevity of model organisms ranging from yeasts to mammals. Importantly, dietary restriction has been shown to extend the health span of primate models. It is thus surprising that little is still known about the molecular mechanisms and signaling pathways that underlie such cellular phenomenon. In this talk, we will present a high-throughput genetic-analysis platform to identify the genes and genetic pathways that mediate lifespan extension by dietary restriction in the budding yeast *Saccharomyces cerevisiae*. Using this methodology, we have characterized in a quantitative manner the chronological lifespan of ~4000 single-gene knockout strains aged under different diet conditions. In doing so, we uncover a group of genes for which dietary restriction aggravates or alleviates the relative lifespan effects of the knockouts. We find that conserved cellular processes such as respiration, autophagy, glycogen accumulation, and control of the cell-cycle underlie the extension of lifespan by dietary restriction. Importantly, an analysis of regulatory targets reveals a defined set of transcription factors that mediate such response, providing a global picture of the gene-regulatory pathways that control lifespan in response to nutrient availability. Our comprehensive analysis of longevity factors provides new insights into the cellular and environmental wiring of aging cells.

# Environmental and biological drivers for viral infection distribution and disease occurrence

**Gerardo Suzán**

Pathogen transmission between animals and humans (zoonosis) are occurring worldwide affecting human and animal health. For instance, vector-borne viruses are responsible for 23 per cent of emerging infectious diseases in humans, domestic livestock and wild animals and complex environmental and biological changes are driving such events.

Several drivers of viral emerging diseases have been identified operating at different temporal and spatial scales. At coarse scales, changes in temperature and precipitation have been associated with changes in the geographical distribution and prevalence of vector-borne diseases transmitted by mosquitoes, ticks, and fleas. These changes can have numerous negative effects, including increased risk of emergence of new pathogens and alteration of transmission dynamics of endemic diseases.

At landscape scale, the expansion of human activities, including deforestation, agriculture, and urbanization, have modified the composition and structure of communities. Habitat fragmentation results in decreased area and increased patch isolation, along with microclimatic and biogeographical changes, altering the richness and the relative abundance of species involved in disease transmission cycles.

Theoretical and empirical evidence also suggests that phylogenetic diversity of hosts, vectors and reservoirs, resulting from modified landscapes, determines the niches available for viruses shaping viral richness and viral diversity. Thus fragmented landscapes, where community and phylogenetic diversity have been modified, are allowing different opportunities for spillover, spillback, and host switching events.

Ecological and evolutionary characteristics of both reservoir and pathogens themselves have been also recognized to play an important role at local scales, being the phylogenetic and behavioral traits drivers for viral distribution and prevalence.

The identification of significant patterns in viral infections may depend on the scale of observation. The scale at which observations are performed is important in understanding underlying patterns. To properly recognize environmental and biological drivers for viral infections, future studies should carefully determine the appropriate spatial and temporal scales for the host-pathogen system under study. Multi-spatial approaches will provide a better understanding on the dynamics of vectors and reservoirs and will have large implications in the control and management of infectious diseases affecting human and animal health.

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# *Aedes aegypti* immune response against dengue virus and other pathogens

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The knowledge of the immune response in the mosquito *Aedes aegypti* is in its early stages and little is known of how mosquitoes recognize and eliminate pathogens including dengue virus (DV). In our research group, we are interested in understanding the cellular and molecular immune mechanisms involved in the elimination and control of DV, with the goal of proposing strategies to interrupt the transmission of this disease. Recently, we have observed that reactive species of oxygen (ROS) and, in particular, nitric oxide (NO) are important to limit viral replication in resistant mosquitoes. When resistant mosquitoes are infected with DV produced large amounts of NO, particularly in the midgut. The enzyme responsible for the production of NO is the nitric oxide synthase (NOS). When the enzyme is inhibited by using L-NAME, viral replication in mosquitoes is produced. In addition, ROS are involved in the induction of systemic immune response in mosquitoes. Recently it has been documented the ability of different species of invertebrates to remember previous encounters with various pathogens. This finding is known as "priming" or trained-immunity and is analogous to the mammalian immune memory. In our group we have determined that the mosquito *Aedes aegypti* retains the information from a previous encounter with non-infective forms of DV (inactive). When mosquitoes were infected (7 or 14 days after the first encounter with the VD), those who were previously treated with inactive DV, avoids virus replication. This ability can be induced since ontogeny (larvae L-4) and is kept in the adult stage inhibiting viral replication. The mechanism responsible of immune memory has not been determined, but our studies indicate that an endoreplication process is activated, with the production multiple copies of immune genes.

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# Cross reactivity between dengue virus and zika viruses: consequences for zika virus pathogenesis and prevention.

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Dengue virus and zika virus are both closely related arboviruses classified into the same family of viruses (*Flaviviridae*) and virus genera (*Flavivirus*). In addition, in urban areas they are transmitted by the same mosquito vectors *Aedes aegypti* and *Aedes albopictus.* In the year 2015, zika fever emerged in Brazil as a disease never seen before in the Americas and from there rapidly spread into more than 40 countries that were already endemic for dengue. Crystallographic studies revealed that the envelope (E) proteins of zika and dengue virus are nearly identical in structure.

The E protein is the viral attachment protein and in the main responsible for the induction of neutralizing and protective antibodies. Thus, it is not surprising that antibodies raised against dengue virus will recognize zika virus and vice versa. It have been reported that antibodies against dengue virus can enhance zika virus infection in FcR receptor (macrophages and dendritic cells) bearing cells *in vitro*. Concurrent or sequential infections with dengue and zika viruses are to be expected in individuals inhabiting endemic regions. The bases for the antibody cross reactivity between both viruses as well possible consequences for the pathogenesis of zika virus fever and prevention will be discussed.

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# THE ZIKA VIRUS: EPIDEMIOLOGY AND PATHOGENESIS

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Zika virus (ZIKV) is the causative agent of the disease of the same name transmitted to humans by mosquitoes of the genus *Aedes*. This virus belongs to the *Flaviviridae* family and genus *flavivirus*. ZIKV causes symptoms in 1 out of 5 infected individuals. The symptoms of zika fever are relatively mild and include fever, headache, joints pain, rash and conjunctivitis. However, the emergency declaration launched by the World Health Organization regarding ZIKV was related with the sharp increase in microcephaly or neurological defects observed in infants born from pregnant women who suffered ZIKV infection and also with cases of Guillain-Barre syndrome associated with ZIKV infection. Epidemiological data and viral replicative cycle studies carried out in cells and in animal models have resulted in the identification of possible mechanisms of vertical and sexual transmission, as well as virus replication in testes and genitourinary tract of women. This infection is a new challenge for public health systems and research groups. The consequences of the ZIKV epidemics in Mexico are not yet fully known but shall be displayed in the next few months.

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# Mitotic bookmarking by TFIIH during zygotic genome activation in *Drosophila* suggests the existence of a short-term transcriptional memory mechanism

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Zygotic Genome Activation (ZGA) allows development to proceed by transcribing the embryo genome. In *Drosophila,* ZGA takes place at the pre-blastoderm embryo during a phase of rapid mitotic divisions. How the transcription machinery is coordinated to achieve this goal in a very short time span is still poorly understood. TFIIH is a component of the basal transcription machinery that is fundamental for transcription initiation by RNA polymerase II (RNAPII). Here, we show for the first time the *in vivo* dynamics of TFIIH components at the onset of transcription in the early *Drosophila* embryo. Interestingly, TFIIH shows a highly oscillatory behaviour between the nucleus and cytoplasm in the pre-Mid-Blastula-Transition (pre-MBT) embryo. From interphase to early metaphase, TFIIH foci are observed, and at prophase, these foci co-localize with the serine-5-phosphorylated form of RNAPII (RNAPII-S5P), suggesting that transcription overlaps with the first mitotic phases in this embryonic stage. Intriguingly, TFIIH, as well as TBP and the RNAPII, remain associated with mitotic chromatin, enriched at the promoters of the histone genes, acting as possible bookmarks for the fast activation of transcription after mitosis in the pre-blastoderm and syncytial blastoderm embryo. Furthermore, we found an essential role for TFIIH core subunits during mitosis in the pre-blastoderm embryo, as mutant organisms show several mitotic phenotypes, including free centrosomes, multipolar spindles and uncompacted DNA. Intriguingly, the RNAPII-S5P was detected at the wild type level in these embryos; thus, a direct role for the core of TFIIH in mitosis cannot be ruled out. However, the transcriptome analysis of these embryos shows transcriptional deficiencies of maternal genes that participate in mitosis in the early embryo. These results provide important insights regarding the role of one of the components of the basal transcription machinery at the pre-MBT, when the zygotic genome is activated in the embryo

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# Genome-wide mapping of tissue-specific enhancers during Drosophila development

**Kasia Oktaba**
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# The long-range promoter interactions landscape of pluripotent cells

Mayra Furlan Magaril

The mammalian genome harbors up to one million regulatory elements often located at great distances from their target genes and is not randomly positioned inside the nuclear space. In the last decade, a group of molecular techniques to study chromosome conformation have been developed allowing the analysis of genome 3D structure at unprecedented resolution. All of these techniques, from 3C (Chromosome Conformation Capture) to HiC are based on measuring ligation frequencies between DNA molecules located in close proximity within the nuclear space. Here I will discuss the results we obtained applying a novel capture method (Capture-HiC) to characterize the promoter long-range interactions genome wide in murine embryonic stem cells and its comparison to the promoter interactome of a commited cell type. I will then briefly mention the current directions of my research as an independent researcher at the Institute of Cellular Physiology (IFC-UNAM).

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# The circadian clock: a biological system at the crossroad of metabolism and epigenetics

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Circadian rhythms govern many important physiological and behavioral functions in almost all organisms. The circadian clock is a time-tracking machinery that allows organisms to anticipate pervasive environmental changes and adapt their physiology to the external time. This control is achieved through a complex autorregulatory network consisting of interconnected transcriptional-translational feedback loops which drive 24hour-period oscillatory expression of clock-controlled genes in a tissue-specific manner. Rhythmic gene expression is assisted by a remarkably plastic circadian epigenome, and chromatin remodelers coordinate with the clock machinery to precisely rhythms on epigenetic marks at circadian genes. This epigenetic regulation further extends to posttranscriptional control of circadian transcription. Interestingly, spatial organization of the chromatin in the nuclear space supports rhythmicity by organizing location of circadian genes in circadian interactomes, where they physically interact in a time-specific manner. Cellular metabolism modulates the circadian epigenome through specific nutrient sensors such as SIRT1, which directly modifies the function of certain chromatin remodelers to modify circadian transcription. Particularly, the histone methyltransferase MLL1 is rhythmically deacetylated by SIRT1, and this event controls its activity. Importantly, availability of SIRT1 cofactor, the metabolite NAD+, has significant implications on SIRT1 subnuclear dynamics and localization.

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# Extracellular small RNAs during parasite-host communication

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The discovery of stable small RNAs moving between organisms, suggests the existence of RNA-based communication, with implications for ecology, agriculture and disease. We have previously reported the presence of exosomes with small RNAs and a worm-specific Argonaute in the secretory products of the parasitic nematode *Heligmosomoides polygyrus*. We are now further characterizing the molecular content of these exosomes, and particularly want to understand the function of the different types of small RNAs during infection.

We performed high-throughput sequencing of small RNA libraries from the exosome and supernatant of *H. polygyrus* secretory products. To aid their characterization, we also used long single-molecule sequencing (PacBio) to improve the genome assembly and annotation of *H. polygyrus*. This allowed us to determine the genomic locations of the secreted small RNAs. Certain classes of RNAs are enriched in exosomes, including short and full-length yRNAs, 22G siRNAs, microRNAs, and specific fragments from tRNAs and rRNAs. Particularly interesting are 22G siRNAs, produced by RNA-dependent RNA polymerases, suggesting that a complex RNA-interference machinery is involved in producing the exosome content. These results will help us understand the functions of exosomes and their cargo during parasitism, and highlight a versatile role for RNA during interactions between organisms.

This project is supported by an HFSP grant (RGY0069).

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# A network approach to drug resistance in breast cancer

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Steroid hormones are involved on cellgrowth,development and differentiation.Such effects are oft en mediated by steroid receptors. One paradigmatic example of this coupling is the estrogen signaling pathway. Its dysregulation is involved in most tumors of the mammary gland. It is thus an important pharmacological target in breast cancer. This pathway, however, crosstalks with several other molecular pathways, a fact that may have consequences for the effectiveness of hormone modulating drug therapies, such as tamoxifen.

For this work, we performed a systematic analysis of the major routes involved in crosstalk phenomena with the estrogen pathway–based on gene expression experiments (819 samples) and pathway analysis (493 samples) –for biopsy-captured tissue and contrasted in two independent datasets with in vivo and in vitro pharmacological stimulation. Pathway based analysis supplemented with probabilistic gene regulatory network inference allowed us to disentangle the molecular origins of pathway crosstalk and its likely relationship to drug-resistance mechanisms.

Our results confirm the presence of a number of crosstalk events across the estrogen signaling pathway with others that are dysregulated in different molecular subtypes of breast cancer.These may be involved in proliferation, invasiveness and apoptosis-evasion in patients. The results presented may open the way to new designs of adjuvant and neoadjuvant therapies for breast cancer treatment

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# Inter-species comparison of endothelial cell gene regulation reveals the conserved control of vascular disease genes

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Comprehensive maps of transcription factor (TF) binding and epigenetic modifications are rapidly generating new testable insights into human disease gene regulation. However, it is still not trivial to identify which gene regulatory regions have the most impact on tissue specific gene regulation. We set out to identify gene regulatory regions that are required for vascular homeostasis by making controlled cross-species epigenetic comparisons (Zoo-ChIP) in aortic endothelial cells (ECs) isolated from multiple species. We profiled the JUN transcription factor, an important member of the AP1 complex, and several histone modifications in human, rat and bovine aortic ECs cultured ex vivo. We identified shared orthologous TF binding and shared orthologous active histone modifications. We found that approximately 5% of JUN and 33% of H3K27ac are shared between human and one additional mammal (rat or cow). These shared putative regulatory DNA regions were enriched for EC pathways including angiogenesis and nitric oxide metabolism. Shared orthologous JUN binding sites also coincided with reported regulatory human disease mutations, several of which have a plausible EC component. Importantly, only a minority of these shared JUN/H3K27ac interactions could have been predicted using established measures of DNA constraint or ChIP-seq signal alone. By comparing our findings to similar studies performed in primary liver tissue, we demonstrate that comparative epigenomic profiling enriches for tissue-specific pathways and distinct human regulatory DNA mutations.

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# Microbial Motility and Random Walkers

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Abstract: In this work I present resent results regarding the motility of *E. coli* and *T. cruzi* in constrained spaced, together with modeling results aimed at characterizing the motility of these microorganisms. Notably, the developed models consider the studied microorganisms as non-Markovian random walkers.

**UNIPARTS**

# Cultivo Celular 3D

Javier Hernández Juárez
UNIPARTS

El cultivo de células ha servido como una herramienta invaluable en la biología celular durante varias décadas. Las monocapas de células con capacidad adherente cultivadas en sustratos bidimensionales (2D) planos y rígidos, tales como poliestireno o de vidrio, han evolucionado como el pilar en sistemas de cultivos celulares convencionales. En el cuerpo, casi todas las células en los tejidos residen en una matriz extracelular (ECM) que consiste en un complejo tridimensional (3D) de arquitectura e interacciones con las células vecinas a través de señales bioquímicas y mecánicas. El cultivo celular 3D emerge como una herramienta que se aproxima de manera eficaz a las condiciones in vivo de un organismo. Las aplicaciones del cultivo celular 3D tienen gran potencial en el futuro de la investigación biomédica, farmacológica y de terapia celular.

**GE HEALTHCARE**

**Usando Análisis Celular Multiparamétrico por microscopia de fluorescencia (HCA) y microscopia de Alta y Súper resolución para resolver preguntas biológicas**

*GE Healthcare Life Sciences*

Resumen:
Recientemente se han desarrollado técnicas de microscopia de fluorescencia para superar las limitaciones de los microscopios convencionales. Conforme las técnicas han ido evolucionando y se han comercializado, el avance de la microscopia de luz ha sido muy aceptado como una herramienta común en los laboratorios que hacen investigación biológica.

Los beneficios del análisis multiparamétrico se aplican a una variedad de ensayos celulares in vivo, como: señalización celular, toxicología, iRNA, diferenciación y morfología celular, ciclo celular, neurología, activación de receptores y más. Esa tecnología permite aumentar el volumen de muestras del ensayo y la productividad, teniendo mejor calidad de imagen y datos haciendo su investigación más amplia y profunda.

Durante este curso se darán a conocer de manera teórica, las bases de estas técnicas, las aplicaciones que permiten y una guía para que usted pueda usar más adelante estas técnicas en su investigación.

**ACCESOLAB**

# Acclaro Sample Intelligence technology: how it works and how sample purity is important for downstream applications

Voula Kodoyianni

Thermo Fisher

DIFFRACTIA MEXICO S. DE R.L. DE C.V.

AVDA. DOS, #26, Int. 6

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**DIFRACCTIA**

# miniPCR: Abriendo las puertas al análisis de ADN

Harvard Medical School

Dana Farber Cancer Institute

Howard Hughes Medical Institute

La amplificación de ADN mediante la reacción en cadena de la polimerasa (PCR, por sus siglas en ingles) ha transformado el mundo. PCR es la técnica central para analizar ácidos nucleicos en laboratorios de investigación, clínica, forense, agricultura, y monitoreo ambiental, entre otras aplicaciones. Más de treinta años luego de su invención, la PCR continua siendo un cuello de botella debido a dificultades de implementación y acceso. miniPCR ha solucionado este problema creando instrumentos de PCR de alta performance y bajo costo. Nuestro fin es expandir el acceso a biotecnología en todos los estratos de la sociedad. Creada en 2013 por científicos latinoamericanos instalados en Harvard, miniPCR esta hoy en uso en cientos de laboratorios alrededor del mundo, y en la Estación Espacial Internacional donde ha conducido la primera amplificación de ADN en orbita espacial. Esta presentación abarcará aplicaciones de la tecnología miniPCR en investigación, educación, e industria, y constituirá el lanzamiento oficial del sistema a cargo de Diffractia México, único distribuidor autorizado en México.

**BIORAD**

# Producción rápida de RNA quiméricos mediante cromatografía semipreparativa

*Adriana Vega Belmont*

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# Effect of pH on *E. coli* producer of a phospholipase A2 from *Micrurus laticollaris* snake venom

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*Se solicita este resumen para presentación oral en el área de Biotecnología*

## Abstract

The growth of *E. coli* on acidified or basified media has been frequently studied in order to discern the molecular mechanisms involved in pH tolerance. Proteomic studies have demonstrated overall strategies for surviving these stressful environments: consumption and export of protons at low pH and proton import and chemo-taxis at high pH. However, the role of cultivation pH on recombinant strains still lacks profound understanding, which is a limitation for improving biotechnology processes. Growing transformed strains of *E. coli* at alkaline pH has proved to alleviate the stress due to permeant weak acids. These molecules are almost unavoidable in aerobic cultures and greatly impair heterologous protein production. The alkaline approach might be advantageous to achieve a higher productivity but the pH stress response and its consequences to the quality of the product must be envisioned. On the other hand, several authors have studied the role of cultivation pH in determining the solubility of the recombinant proteins, although with inconsistent results. A recent report from our laboratory demonstrated that specific pH conditions influences the physical-chemical properties of inclusion bodies. These aggregates are the major bottleneck in recombinant systems, but their alteration is relevant to increase the efficiency of recombinant protein production. Therefore, in this work we aimed to compare the effects of uncontrolled pH and pH 6.5, 7.5 and 8.5 on the growth and recombinant protein production of an *E. coli* strain producing a phospholipase A2 (PLA2) from the venom of the Coral snake (*Micrurus laticollaris*).
Our results show that the growth and substrate consumption kinetics are similar in all cultures. At pH 6.5 and 8.5 the biomass was ~10% lower than at 7.5 and without pH control. The biomass yield was 20% lower for growth at pH 8.5 with respect to the other strategies and the highest specific productivity was obtained at pH 7.5. These differences are probably associated with a stress response, because of extreme pH or diffusible organic acids. In all cases, the recombinant protein was completely recruited to inclusion bodies at 10 h after induction, which is explained by its high aggregation propensity, previously evaluated *in silico*. Following these results, we evaluate the effects of pH in the inclusion bodies obtained in these cultures, to describe the cellular responses associated pH changes and the characteristics of the environment that promote the phospholipase A2 aggregation. The analysis of the aggregates using FTIR showed differences in their secondary structure content, suggesting a variation on protein synthesis by external pH.

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# Biodegradation of Diclofenac by the native fungi *Pleurotus djamor* isolated from Chiapas

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Abstract

Diclofenac is an anti-inflammatory nonsteroidal used in humans and animals to reduce the symptoms of pain and inflammation. Due to its widespread use has a steady income to the environment, it is found in several waterbodies; causing a potential impact on populations of aquatic organisms. Therefore, various processes have been used for the removal of diclofenac in the aquatic environment. The white rot fungi produces ligninolytic enzymatic complex such as laccase, phenol oxidase and magneso peroxidase, which are relatively non-specific and the use of free radical allow them catalyze the degradation of a wide variety of contaminants in the environment. In this paper the ability of *Pleurotus djamor* in the biodegradation of diclofenac was evaluated in liquid medium. Biodegradation was performed after 5th and 7th day of micelial growth in 125 ml Erlenmeyer flasks in a total volume of 30 ml in malt medium. The initial drug concentration was 10 mg / L obtaining 83% and 43% biodegradation within 30 min of reaction, respectively; while biodegradation reached after 6 hours of incubation was 93% and 90%, respectivelly. Indicating that the 5th day of growth reaches high percentages of degradation. The results obtained showed that the fungus Pleurotus djamor effectively degrade diclofenac.

Keywords: Diclofenac, *Pleurotus djamor*, biodegradation, ligninolytic enzyme.

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# The Defensin from Avocado (*Persea americana var. drymifolia*) PaDef Induces Apoptosis in the Leukemia Cell Line K-562

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Cancer caused 8,2 million deaths in 2012 in the world. The leukemia is including in the ten most important types of cancers for its incidence and mortality. The conventional treatments to cancer are surgery, radiotherapy and chemotherapy; however, these approaches have a low therapeutic index and severe side effects. These limitations have led to the search for new alternative therapeutics such as vaccines and natural products. An attractive alternative is the use of antimicrobial peptides, or AMPs, which represent a novel family of anticancer agents that avoid the limitations of conventional treatments. Today, there have been described 2717 AMPs (http://aps.unmc.edu/AP/main.php), from these only 196 showed anticancer activity. In plants there are 3 families of AMPs with cytotoxic activity: defensins, thionins and cyclotides. However, the mechanisms of cytotoxicity are not known in detail. The aim of this study was to evaluate the cytotoxicity of defensin PaDef from avocado (*Persea americana* var. *drymifolia*) against leukemia line K562. The AMP PaDef (47 aa) was chemically synthesized; the cell viability was evaluated by MTT assay and flow cytometry. The determination of apoptosis (Annexin V/7AAD), mitochondrial $\Delta\Psi m$, membrane potential and intracellular calcium release were analyzed by flow cytometry. Finally the caspase 8 and 9 activities were determined by luminescence. PaDef inhibited the viability of K562 cells in a concentration-dependent manner, with an $IC_{50}$ = 97.3 µg/ml. Interestingly, this concentration was not cytotoxic for PBMC from healthy donors. The viability of peripheral blood mononuclear cells, used as control, was unaffected by this AMP. PaDef induced apoptosis in K562 cells, however did not affect the mitochondrial $\Delta\Psi m$, membrane potential and intracellular calcium release. On the other hand, caspase 8 and 9 activities were induced by PaDef treatment. In conclusion, these results indicate that the AMP PaDef is cytotoxic to K562 cell line inducing extrinsic apoptosis, which is a novel property for a plant defensin.

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# Soil and rhyzospheric bacteria with inhibition of root rot causing pathogens in chili pepper, with differential SAR related gene induction in plant: nine partially sequenced genomes.

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The consumers worldwide are pressing to diminish the pesticide use in agriculture, and at the same time in favor of natural tools as biocontrol to fight the phytopathogens. The nature and variability of the molecular mechanisms in the biocontrol agents involved in the inhibition of phytopathogens, the regulation of the friendly plant-microorganism interaction and the promotion of plant growth is still limited. The object in this work is to aisle soil and rhyzospheric bacteria from wild plants whith the capacity of inhibition against four pathogens causal agents of root rot in chili pepper, and the sequencing of some genomes of bacteria with friendly interaction in plant and others with hostile behaviour, to desciphere some molecular specific mechanism in support of the distinctive bacterial performance. For this pourpose, 94 bacterial isolates from soil or rhyzospheric origin were challenged against virulent isolates of *Phytophthora capsici*, *Fusarium solani*, *Fusarium oxysporum* and *Rhizoctonia solani* and in the outsanding bacteria isolates with 60% or higher pathogen inhibition, it was assessed the indolacetic acid and siderophore production; furthermore these bacteria were inoculated in the root in pepper plantlets to identify these with friendly or hostile in plant interaction. A collection of 26 bacteria isolates are prominent in the inhibition against one or more pathogens and in the indolacetic acid production; from these, 13 offer friendly interaction in pepper plantlets and induce differentially two systemic acquired resistance (SAR) related genes. Four isolates outstand with polyvalent capacity of inhibition against the four pathogens in addition to friendly interaction in plant, also inducing differentially the SAR related genes, two producing indolacetic acid and siderophores. In nine partially bacterial sequenced genomes the average genome size is 4.5 MB, the average GC content is 45.5% except in a bacteria without inhibition capacity to any pathogen where this percent ascend to 68.3. Comparative genome analysis is underway.

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# Oxygen transfer rate affects undecylprodigiosin synthesis in *Streptomyces lividans*: relation with recombinant glycoprotein production

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Oxygen availability is a key parameter in aerobic bioprocess. It could be measured as oxygen transfer rate (OTR) that is given by the product of the $k_La$ and the oxygen gradient concentration in the liquid interphase. OTR can be related to the metabolic state of a microorganism (Kunze et al., 2012).
On the other hand, *S. lividans* is an aerobic filamentous bacteria that is widely used for the production of recombinant glycoproteins and antibiotics (Ong et al., 1994, Lara et al., 2004, Gamboa-Suasnavart et al., 2011). In the *O*-mannosylation pathway GTP is required to activate manose that is mainly formed from fructose 6-P. Differences in *O*-manosylation degree have been found related to culture conditions in shake flask (Gamboa-Suasnavart et al., 2011). These conditions could be separated in two phenomena: hydrodynamics and aeration (measured as OTR).
*S. lividans* presents a complex secondary metabolism in which mainly actinorhodin and undeylprodigiosin (UDP) are mainly produced. In the biosynthetic pathway of UDP, NADH, carbon and GTP are consumed (Cerdeño et al., 2001). In this work, we evaluated the relation between OTR and UDP production and its impact on *O*-mannosylation degree in three shake flask designs: Normal Erlenmeyer Flask (NF), Baffled Flask (BF) and Coiled Flask (CF) and in bioreactor at three controlled OTR values (1.5, 9.0 and 18.0 mmol/lh).
OTR was measured in shake flask, showing a maximum of 9.16±0.15 and 9.36±0.28 mmol $l^{-1}$ $h^{-1}$ in CF and BF, respectively, and 0.66±0.48 mmol $l^{-1}$ $h^{-1}$ in NF. UDP production increased 15 folds in NF 1.54 ± 0.02 mg/L, 0.004 ± 0.001 and 0.1200 ± 0.002 mg/L, of undecylprodigiosin were obtained in NF, BF and CF, respectively. In bioreactor the same trend was observed. 0.124 ± 0.004, 0.0061 ± 0.001 and 0.002 ± 0.002 mg/L were found in 1.5, 9.0 and 18.0 mmol/lh respectively.
Changes in O-mannosylation degree were found. At low OTR values only 2 manoses were found in APA protein C-terminal peptide, and up to 5 manoses in Higher OTR values. UDP overproduction could lead to these differences in O-mannosylation degree.

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# Engineered Cytochrome P450 BM-3 reductase domain stabilized by consensus mutagenesis

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The catalytically self-sufficient cytochrome P450 from *Bacillus megaterium* (BM-3) is of great interest from an industrial point of view; however the low stability of its reductase domain limits its application. In the present work we describe the stabilization of the di-flavin reductase domain of BM-3 using a consensus mutagenesis approach. Upon phylogenetic analysis of closely related CytP450s (% identity higher than 38%), a total of 14 amino acid residues was targeted for mutagenesis and evaluated in terms of their stabilizing effect relative to the wild-type reductase domain. Recombination of the six most stabilizing mutations resulted in the identification of thermostable variants with up to 10-fold longer half-life at 50 °C. Spectroscopic characterization of two selected variants indicated that the mutations increased the thermal stability of the FAD-binding domain. Activity studies revealed that the optimal temperature ($T_{opt}$) has shifted from 25 °C in the parental enzyme to 40 °C for the best variant. This variant was determined to exhibit a more cooperative folding and higher electron transfer efficiency at elevated temperature. Characterization of the different reductase domain variants allowed for the discrimination between mutations that provide more resistance against thermal inactivation and those that, in addition to thermostabilization, contribute to the retention of catalytic activity at elevated temperature.

# Some like it hot: Biomolecular analytics using MicroScale Thermophoresis.

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#NanoTemperTechnologiesServiçodeTecnologíadoBrasil, Av. Andrômeda 2162, Sala 02. SãoJoseDosCampos, SP §NanoTemperTechnologiesGmbH, Floessergase 4, Munich MicroScale Thermophoresis (MST) is a powerful technique to quantify biomolecular interactions. It is based on thermophoresis, the directed movement of molecules in a temperature gradient, which strongly depends on a variety of molecular properties such as size, charge, hydration shell or conformation. Thus, this technique is highly sensitive to virtually any change in molecular properties, allowing for a precise quantification of molecular events independent of the size or nature of the investigated specimen. When performing a MST experiment, a temperature gradient is induced by an infrared laser. The directed movement of molecules through the temperature gradient is detected and quantified using either covalently attached or intrinsic fluorophores. By combining the precision of fluorescence detection with the variability and sensitivity of thermophoresis, MST provides a flexible, robust and fast way to dissect molecular interactions.

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# Design of Multi-domain Proteins for Artificial Virus-like Supramolecular Nanoparticles

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Building upon the current knowledge about virus structure and self-assembly we aimed to design proteins that imitate viral self-assembly to produce well-defined artificial viral-like nanoparticles (VLPs) with cell transfection capabilities. After identifying biochemical functionalities in capsid proteins need to form VLPs we hypothesized that encoding all those functionalities in an artificial polypeptide sequence will form VLPs. Through recombinant production in *P. pastoris* yeast we produced a family of proteins called "C-$S_n$-B" carrying three different domains, each one performing the following functionalities: colloidal stability ("C"), self-assembly ("$S_n$") and electrostatic nucleic acid binding ("B") (Figure 1a).

Upon binding to single DNA templates, the proteins self-assembles cooperatively around the dsDNA template, packing it into monodisperse and highly ordered elongated VLPs (Figure 1b), following a similar mechanism as Tobacco Mosaic Virus. Also, the VLPs protect nucleic acids against enzymes and deliver them into cells. This is a proof of concept that we could design minimal artificial viral systems, which could be harnessed to better understand the self-assembly mechanisms of natural viruses, as well as to program systems for controlled delivery of therapeutic nucleic acids and drugs and for multivalency display for nanovaccines, immune-modulation and anti-microbial nanomaterials.

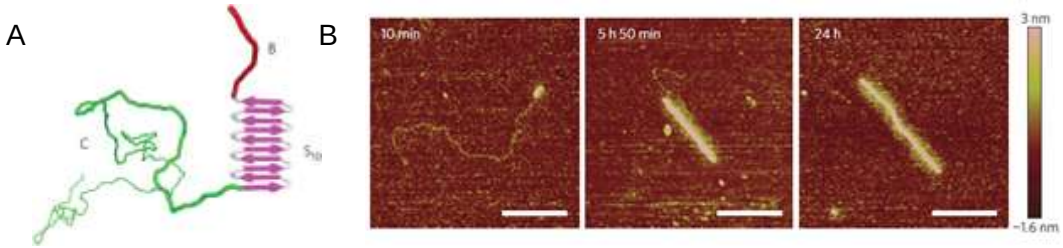


**Figure 1**. A) Design of a minimal viral coat protein C–S*n*–B. B) Atomic force microscopy snapshots at different times of formation process of VLPs. Bar scale is 200 nm.

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# Experimental strategies to enhance production and activity of laccase from *Therrmus thermophilus*

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Laccases belong to the family of blue multicopper oxidase. These enzymes are characterized by the presence of a cluster of four copper atoms (type 1 copper; type 2 copper and two type 3 copper atoms). Laccase catalyses the four electron reduction of $O_2$ to $H_2O$ by transfer of electrons from the substrate. These enzymes have a wide substrate range, can oxidize any substrate with characteristics similar to *p*-diphenol, such as: diphenols, aminophenols, polyphenols, and lignin.

Laccases have been isolated in higher plants, fungi, insects, yeasts and bacteria. This enzymes attracted considerable interest for applications in many fields of industrial and environmental process: biobleaching, xenobiotic bioremediation, industrial waste treatment. One of the prerequisites for an enzyme to be applicable in industrial processes is thermostability or thermotolerance. The most thermostable laccases have been isolated from bacteria, being active in extreme conditions, pH 3-9, temperature 25°C-90°C, and have been cataloged “easy protein expression. In *Thermus thermphilus* has been studied the laccase Tth-laccase, this enzyme is the most thermostable laccase than have been reported. The $T_{1/2}$ from Tth-laccase is 14 h at 80°C and its optimal activity is under following conditions: temperature 90°C, pH 4.5, using substrate 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). Tth-laccase offers an attractive alternative in industrial processes because their biochemical proprieties. However, a limitation of this enzyme is their low production and activity compared to fungal laccase. In the present work we designed experimental strategies to enhanced Tth-laccase activity and production. There are many reports where modified conditions to enhance enzyme production in *E. coli*: pH, temperature, expression time and incorporation of the tags based in synonyms mutations, because they don’t change the protein but they change the expression levels. Here we expressed Tth-laccase in *E. coli* and designed N-terminal targets to enhanced its production, and obtained a significantly higher production in comparison with Tth-laccase wild type. Tth-laccase expressed in presence of copper resulted in an increase in activity. These strategies may be considered for application of the Tth-laccase in the industry having a considerable impact in its application.

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# pH effect on growth and lipids accumulation in *Chlamydomonas reinhardtii*

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Microalgae are photosynthetic microorganisms that confront stress conditions, such as salinity, nutrient starvation, metal toxicity heavy, light irradiation, temperature, pH fluctuations, among others, exhibiting changes in the lipids metabolism, mainly as triacylglycerides (TAGs) accumulation. These kinds of lipids serve as a protective mechanism against stress conditions. Due to the above, microalgae are considered as alternative for improving the biodiesel production which is obtained through transesterification reactions of TAGs. *Chlamydomonas reinhardtii* is an important study model with some advantages (fast growth, whole-genome sequence, generation of stable transgenic lines over short periods, growth in containers that reduce environmental pollution, facultative autotrophic and heterotrophic organism and easy genetic manipulation) that have allowed analyze their molecular and biochemical mechanisms in lipids metabolism pathway under stress. Therefore, the objective in this study was to evaluate the effect of pH (7.8 and 8.5) on the growth, lipids accumulation (qualitative and quantitative) and transcriptional profile of some genes involved in lipid biosynthesis in *C. reinhardtii.* According to our results, the $WT_{Ws}$ control and the $WT_{pH7.8}$ cultures reached the stationary phase at 96h and 108h respectively, while the $WT_{pH\ 8.5}$ culture showed a slow growth; in this pH, we also observe a decrease in chlorophyll content (18.352 µg/gr) compared with the 231.2 and 404.9 µg/gr obtained in the $WT_{Ws}$ and $WT_{pH\ 7.8,}$ respectively. Related with the lipid content, the control culture ($WT_{WS}$) reached a 4.97% of dry cell weight (DCW), while in the stress conditions, the total lipid content increased 2.99 (14.81% DCW) and 2.311(11.43% DCW) times in the $WT_{pH\ 7.8}$ and $WT_{pH\ 8.5}$ cultures, respectively. The qualitative analysis revealed a significant accumulation of lipid droplets in the $WT_{pH\ 7.8}$ culture compared with the $WT_{ws}$ control. The qRT- PCR analysis showed a significant accumulation from some transcripts, including *β-carboxyltransferase* (*BCX1*), *acyl carrier protein* (*ACP1*) and *acyl-ACP thiolase* (*FAT1*) involved in fatty acids biosynthesis in response to stress by pH 7.8. These evidences suggest that pH stress (7.8) positively affects the lipids content.

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# Padlock probe-rolling circle amplification assays for the detection of high risk human papillomaviruses

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Every year more than half a million women are diagnosed with cervical cancer. The causal factor for cervical cancer is a persistent human papillomavirus (HPV) infection with 'high risk' (HR) HPV types. HPV-16 and -18 are associated with 70% of cervical cancer cases worldwide. Among the HR types, HPV-33 is considered the most prevalent (33%) in cervical infections in the state of San Luis Potosi and HPV-45 is associated with 50% of cervical cancer cases in this region. Currently, the methods most used for HPV DNA detection are hybrid capture and PCR. Padlock probes (PP) are single-stranded linear oligodeoxynucleotides that detect target sequences with very high specificity and selectivity. The recognition sequences of the PP are located at both the 5'- and 3'-ends and connected by an intervening sequence encoding a specific restriction site. When hybridized to a target molecule the two ends of the PP are juxtaposed and can be covalently joined by ligase to form a single-stranded circular template. This product can be amplified by rolling circle amplification (RCA) with Phi29 polymerase in isothermal conditions. PP-RCA assays are a fast, sensitive alternative to PCR without a thermocycler, suitable for diagnosis at the point of care. We designed type-specific padlock probes of different length: PP-HPV16, 96 nucleotides (nt); PP-HPV18, 114 nt; PP-HPV33, 132 nt; PP-HPV45, 151 nt, based on the sequences of the corresponding ORF E6 sequences with a *Bam* HI restriction site on the intervening sequence. The templates used for the assays were DNA samples from HPV cervical infections (HPV-16, -18, -33, -45). PP-RCA assays are performed in five steps: 1) denaturation of the target sequence, 2) probe hybridization and ligation, 3) amplification of the circularized probe as concatemers formed by double-stranded monomers in tandem, 4) restriction of the concatemers and 5) electrophoretic analyses of the monomers. With our designed type-specific PPs we have obtained the expected monomers using as templates plasmids carrying complete genomes of HPV-16, -18, -33 and -45 as well DNA from cervical samples with infections by the corresponding viral types. This technique is suitable for the development of a rapid, sensitive and 'on-site' HPV infection diagnosis. We are currently working to develop a multiplex PP-RCA assay for the four HPV types.

# Exploring PD-1 and PD-L1 conformational landscape using molecular dynamics simulations.

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Abstract:

In recent years the PD-1/PD-L1 immunologic checkpoint has appeared as an extremely attractive target for the treatment of melanoma, non-small cell lung cancer and genitourinary cancers. In former years, the development of inhibitory molecules targeting this pathway was hindered by the insufficient structural information available. However, this scenario has changed with the publication of human PD-1/PD-L1 complex x-ray crystal structure. In the talk are exposed the structural bases for the interaction, the plasticity of the binding partners upon complex formation and the evaluation of conformational landscape in a search for drugable binding pocket. This is accomplished by using Molecular Dynamics simulations on the protein complex and its individual elements. The results provides a rational from which new pharmacophoric hypothesis can be stablished.

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# Induction of IL-10 and TGF-β in intestinal mucosa of rat by Lactococcus lactis IL-22 secretor. An alternative treatment of experimental liver cirrhosis.

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Intestinal microbiota is involved in the regulation of inflammatory processes in the intestine and systemic chronic degenerative diseases, such as liver fibrosis, since it induces the release of anti-inflammatory cytokines in the intestine inhibiting the transcription NFκβ factor thus, decreasing the synthesis of pro-inflammatory cytokines (Gomez et al, 2011). In monocytes/macrophages and dendritic cells, IL-10 inhibits the production of pro-inflammatory cytokines and chemokines. Also inhibits the expression of molecules of the major histocompatibility complex (MHC or HLA) and co-estimulatory as CD80 and CD86 of dendritic cells and macrophages.
The anti-inflammatory effects are also reflected on the blockade of proinflammatory TH1, TH17 and TH17 + cells. It also has a direct effect on CD4+ Foxp3+ Treg cells to promote their survival (Banchereau et al., 2013). As a therapeutic strategy, it has been administrated IL-10 in rats with cirrhosis induced by CCl4 showed decrease the expression of collagen type I, TNFα, TGFβ, MMP-2 and TIMP-1 in hepatic stellate cell (HSC) (Huang et al., 2006; Zhang et al., 2007).
Another important anti-inflammatory cytokine is IL-22 that is involved in the control of bacterial infections by overexpression of antimicrobial proteins; also promotes tissue repair through the survival and proliferation of liver cells (Kong et al., 2012). The anti-fibrotic effect of IL-22 is achieved through STAT3 activation which in turn induces senescence HSC (Kong et al., 2013). Ki et al. (2010) evaluated an adenoviral vector to direct the gene of IL-22 in mice with liver injury induced by alcohol, founding hepato-protective effect by decreasing the expression of related steatosis genes, and increased expression of antioxidant genes, anti-apoptotic and anti-microbials.
In this work was evaluated by immunohistochemistry the presence of IL-10 and TGFβ producing cells in Peyer's patches and in lamina propria of intestine colonized by recombinant Lactococcus lactis secreting IL-22 (Loera-Arias et al cells producing IL-10, 2014) in a model of fibrosis induced with CCl4 in Wistar rat and the expression of IL-10. These observations allowed us to detect a decreased liver fibrosis induced in rat model.
Our results indicated that the recombinant strain of Lactococcus lactis is a viable therapeutic strategy for the release of anti-inflammatory cytokines (IL-10 and TGFβ), since in cirrhotic tissue overexpression of 20% was found compared to the healthy intestine; while that in our groups: doxazosin with recombinant Lactococcus l., and recombinant Lactococcus l., was found overexpression at least 50% compared to healthy intestine, which supports our proposal previously discussed on possible treatment for liver cirrhosis.
With this work, we conclude that recombinant Lactococcus lactis is a good secretor treatment of IL-22 which proved to have biological activity based by promoting the expression of inflammatory cytokines (IL-10) which was reflected in our results.

# Effect of aging in lungs of normal and bleomycin-induced fibrosis analyzed in wild type and accelerated aging *Zmpste24* deficient mice.

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Idiopathic pulmonary fibrosis (IPF) is a progressive and lethal age related disease, characterized by aberrant epithelial cell activation and an exaggerated extracellular matrix accumulation in the interstitial space. The prevalence/incidence of IPF increases with age, however the mechanisms linking aging and IPF are uncertain. Experimental models of lung fibrosis in "normal" aging mice are scanty mainly because these animals are time-consuming and expensive. Recently, it was demonstrated that deletion of the gene *Zmpste24* in mice causes an accelerated aging phenotype (including scoliosis, muscular dystrophy and dilated cardiomyopathy). Thus, we aimed to evaluate the lung response under basal and bleomycin induced fibrosis in premature aging mice. We used Zmpste24 deficient mice (KO) at 4 weeks ("young") and 16 weeks ("old") and compare them with young (4 weeks) and old (82 weeks) wildtype (WT) mice. Under basal conditions, analysis of lungs of both, aging wildtype and *Zmpste24* deficient mice showed increased hydroxyproline content as well as increased α-smooth muscle actin. The increased collagen fibers were localized surrounding the airway walls. After bleomycin oropharyngeal administration, lungs of young WT and KO mice, showed increased collagen content and similar fibrotic lesions. Old WT mice developed a significantly higher response to bleomycin. Surprisingly, aging KO mice did not developed a fibrotic response to bleomycin. Decreased MMP2 gene and protein expression were found in lungs of old KO mice. These findings suggest that old *Zmpste24* deficient mice, do not respond to bleomycin-induced fibrosis as old WT mice.

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# CHANGE IN PROTEIN-LIGAND SPECIFICITY THROUGH STATISTICAL COUPLING ANALYSIS.

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**Abstract**

Protein-ligand specificity change is a hard problem to tackle through rational design. One of the main reasons of this is the complexity to see the role of residues to a new phenotype before detailed experimental approaches.

The first attempts for PBPs computational designs to bind ligands have been under big discussion. Besides, the problem regarding designs with PBP scaffolds is not delimited to finding the sites and proper mutations that could favor binding of novel ligands, but also avoiding that such mutations compromise the stability and/or the conformational changes needed in the PBP closing upon ligand trapping.In this work, we show that combining the Statistical Coupling Analysis (SCA)[1] of two periplasmic binding protein groups, which differ at the ligand specificity, is enough to identify sites which transform LAO, a periplasmic binding protein able to only bind positive amino acids[2], to now, being able to bind mainly glutamine. After an analysis of the phenotypes from the first round of point mutations proposed, it was possible to achieve the glutamine-binding phenotype with just one point mutation in LAO sequence.

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# Comparative study of transport and assimilation of xylose in conventional and non-conventional yeasts

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D-xylose is the second most abundant fermentable sugar in lignocellulosic waste. It is converted by several bacteria, yeasts and fungi mainly into ethanol. Within this metabolic pathway, there are several by-products of high biotechnological interest. Xylitol, a five carbon polyol, is widely used in pharmaceutical, dental and food industries. It is currently obtained by chemical hydrogenation of D-xylose using Ni-catalyst at high temperature and pressure which makes it an expensive process. Microbial production of xylitol is being encouraged as a potential alternative to satisfy such economic demand. Its productivity has been reported as limited by several factors such as: redox imbalance, low or null expression of enzymes involved in metabolic pathways, and inefficient xylose transport systems. The model yeast *Saccharomyces cerevisiae* is uncapable of transporting xylose into the cell and metabolize it due to lack of enzymatic machinery for such process. Several non-conventional yeasts have been studied for its xylose-transport proteins and enzymes (*Candida guilliermondii* and *Pichia Pastoris*) in order to clone their genes into strains of *S. cerevisiae* for a higher ethanol production. Known strains for high xylitol yield (*Debaryomyces hansenii*, *Candida sp*.) are used for extraction and purification of enzymes and redox stabilizers. The aim of this study was to compare the transport of xylose at different pH values (4 and 6) in order to elucidate whether it is carried out by a sugar-symporter or a facilitated diffusion system; and its further transformation into xylitol by different wild-type strains: *C. guilliermondii* NRRL Y-2075 USDA, *S. cerevisiae* ITM-2014, *D. hansenii* ATTC Y-7426, *Kluyveromyces marxianus*, *Zygosaccharomyces bailli* and *Clavispora lusitaniae*. Also experiments were performed in which changes of the pH of the incubation medium were followed upon the addition of xylose or glucose. Hitherto, *C. guilliermondii* showed the lowest Km value for xylose transport (1.1 μM, pH 4; 1.6 μM, pH 6). There was no evidence of a xylose/$H^+$ symporter in any of the strains based on their ability to alkalize the environment when fed with xylose after growth on xylose and starvation for 12 h. As for xylitol production, in 36 hours none of the strains consumed more than 50% of the xylose available (20 g/L), as a result, low xylitol yields were obtained (up to 0.01 g/L/h), not comparable with ethanol production (0.1g/L/h). *C. guilliermondii* was also the best xylitol producer which comes as a result of its ability to stabilize the redox imbalance in the first steps from the xylose metabolic pathway.

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# Analysis of the Redox Components of *Bacillus subtilis* cytochrome $b_6c$ Complex. The Activity of a Menaquinol:cytochrome *c* Reductase.

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*B. subtilis* is a Gram positive bacterium, generally harmless to humans. It can grow aerobically or anaerobically, either by using nitrate or nitrite as a terminal electron acceptor, or by fermentation.

The *bc*-type complex of *B. subtilis*, is called a $b_6c$ complex, since it shares characteristics of $bc_1$ and $b_6f$ complexes. The operon *qcr* (for quinone:cytochrome *c* reductase) encodes a three-subunit complex. Two of these genes *qcrB-C* encode a split cytochrome *b*. QcrB, a $b_6$ cytochrome, has four transmembrane domains, and four conserved histidines that coordinate the two hemes and a cysteine-43 homologous to Cys-35 that binds heme *x* covalently (as in photosynthetic $b_6$). QcrC is equivalent to the photosynthetic subunit IV, it has three transmembrane domains homologous to the last three carboxy-terminal domains of cytochrome *b* and a *c*-type cytochrome attached to the C-terminus of the protein. An iron-sulfur protein (QcrA) completes the redox subunits of the complex.

Since the $b_6c$ complex oxidizes naphtoquinol, to measure its activity with reduced naphthoquinones is problematic due to their low redox potential (-74 mV), and their high rate of autoxidation. Special conditions have to be set in order to maintain these substrates reduced (prepared immediately before use, acidified solvent, nitrogen atmosphere, etc).

Several studies have shown that it is possible to measure activity of respiratory complexes by using benzoquinones or synthetic naphthoquinones that can be incorporated into the membrane. However, they only report that the naphthoquinones are rapidly auto oxidizable compared to benzoquinones and prefered low pH buffers to avoid autoxidation. But the chemical reduction (cytochrome *c* directly reduced by naphtoquinol) is not addressed.

Here, we characterize the $b_6c$ complex of *B. subtilis* in three aspects: one, the succinate: cytochrome *c* reductase assay with eleven naphthoquinones and compared the activity obtained to the activities obtained with the endogenous quinone or with the quinol analog decylubiquinol in isolated membranes. Second, with redox titrations to obtain the $E_m$ of the $b_6c$ cytochromes, and third, by monitoring the kinetics of the $b_6c$ complex cytochromes in an enriched fraction of the $b_6c$ complex with a stop flow. Here we propose the first approach to the menaquinol:cytochrome *c* reductase Q cycle.

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# BIOLOGICAL ACTIVITY OF EXTRACTS *Tournefortia spp*. CELL LINE MCF-7 BREAST CANCER AND HeLa OF CERVICAL CANCER.

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**SUMMARY**

Cancer is the second leading cause of death in developed countries due to the increased in its incidence, there is a great medical and social interest in this disease [1]. In Mexico in 2010, the main malignant tumors in the adult female population were breast cancer (24.3%) and cervical (9.7%) [2]. Plants have played an important role as a source of anti-tumor agents more than 67% are derived from plants, marine organisms and microorganisms [3]. The Boraginaceae family has species economically important, mainly as medicinal plants [4], has been observed anti-tumor activity in the genus Arnebia (HL-60, HeLa and MCF-7) [5]; *Lithospermum* (B16F10) [6]; *Onosma* (SBcl2, WM35, WM9, and WM164) [7]. In this work the biological activity of the aqueous extract was evaluated Tournefortia spp. (Boraginaceae) in the cell lines MCF-7 and HeLa cervical cancer and breast cancer, respectively. *Tournefortia* spp, is commonly known as cancer or grass black grass in the central valleys of Oaxaca, and used in traditional medicine to treat different diseases, including cancer; therefore interest in assessing the in vitro effect of plant extracts.

For this work, the chloroform extract sheet and then two partitions, the first with methanol and then with water (50g tissue 500ml solvent) was performed by the technique of maceration for 24 hours, each partition obtained was dried with nitrogen gas and stored at 4 ° C for later use. The partition the aqueous extract in cell lines MCF-7 and HeLa at different concentrations (2.5, 5, 10, 20, 40 and 80 µg/ml) for 48 hours at 37 ° C, 5% $CO_2$ was evaluated, subsequently they performed MTT assays and Crystal Violet for inhibition of metabolic activity and cell viability. FDA testing and hematoxylin-eosin were also performed to elucidate whether the extract induces cell death by apoptosis.

When analyzing the results of the effect on the cell line MCF-7, the following percentages of inhibition of metabolic activity was observed 18.45% with 2.5 µg/ml, 20.9% with 5 µg/ml, 21.1% with 10 µg/ml, 31.6% with 20 µg/ml, 35.3% with 40 µg/ml and 35.7% at 80 µg/ml; percent inhibition of cell viability were 7, 15.2, 16, 21.4, 36 and 39.3% at the same concentrations.

Regarding the effect of the extract on the HeLa cell line cervical cancer, the following percentages of inhibition in metabolic activity were observed: 1, 2, 8, 14, 21 and 23%; percent inhibition of cell viability were 0, 2, 7, 11, 14 and 19% in the aforementioned concentrations.

The aqueous extract Tournefortia spp. showed antiproliferative activity in cell lines MCF-7 and HeLa cells in a dose-effect relationship, showing the highest percentages of inhibition at 80 µg/ml.

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# 17-DMAG disturb the subcelular and extracellular localization between Hsp90α and Hsp90β and determines the cell migration capacity in cervical cancer cells

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**Introduction.**The Hsp90 family is composed of two major cytosolic isoforms in mammalian cells, Hsp90α and Hsp90β. Hsp90 isoforms are involved in the folding, activation, and stabilization of numerous proteins in both physiological and disease conditions. The specific functional role of each isoforms remains poorly understood, particularly their involvement in diseases such as cancer. In this regard, we recently demonstrated that Hsp90α and Hsp90β have a differential role on the Akt kinase activity. In turn, Akt is a major positive regulator of the β-catenin signaling pathway, playing a key role in the transcription of genes involved in proliferation and cell migration in both normal and cancer cells. Recently, Hsp90α and Hsp90β isoforms have been associated to degradation of extracellular matrix by activating client proteins such as MMP2 and MMP9. To date, several pharmacological inhibitors of Hsp90, including to 17-DMAG, have been tested for the treatment of cancer, however, the efficacy in their antitumor effects is limited. In this regard, we propose that the different subcellular and extracellular localization of the Hsp90 isoforms could be responsible of this poor efficacy, and the malignancy of cancer cells. **Aim**. To evaluate the role of the subcellular and extracellular localization of Hsp90α and Hsp90β on cell migration mediated by Akt/β-catenin, in cervical cancer cells treated with 17-DMAG. **Material and Methods.**We performed wound migration assays with dose-response curves for 17-DMAG inhibitor on cervical cancer cells (HeLa and C33A), and supervised these experiments by time-lapse video-microscopy. In addition, we analyzed the expression and subcellular and extracellular localization profiles of Hsp90α and Hsp90β by western blot, immunofluorescence and the co-localization and interaction pattern Hsp90α and Hsp90β with Akt and β-catenin by co-immunofluorescence and co-immunoprecipitation. **Results.**We found that the treatment with 17-DMAG in cervical cancer cell lines induces an inversion of the expression profile between Hsp90α and Hsp90β isoforms modifying the invasive phenotype. In HeLa cells, 17-DMAG induces loss of extracellular and nuclear localization of Hsp90α, along with loss of the nuclear β-catenin. This effect was associated with the abundance of Hsp90α expression, before of treatment, and with the susceptibility to anti-migratory effect of 17-DMAG. In contrast, in C33A cells, 17-DMAG promotes the nuclear co-localization of nuclear β-catenin with Hsp90α associated to presence of extracellular Hsp90α, providing resistance to the anti-migratory effect of the 17-DMAG. **Conclusions.**In this study, we described for the first time the differential role of the nuclear subcellular and extracellular localization of Hsp90α and Hsp90β on cell migration associated to the activation of β-catenin in cervical cancer cells.

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# Semi-automatized analysis using image-based flow cytometry to study capacitation-associated increase in tyrosine phosphorylation in human sperm

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## ABSTRACT

After ejaculation in the female tract, the human sperm must undergo a maturation step known as capacitation in order to acquire fertilizing capacity. This process includes the development of hyperactivated motility and the preparation for the acrosomal reaction. At molecular level, capacitation is associated with changes in plasma membrane composition, ion concentrations, an increase in protein tyrosine phosphorylation, among others.

Protein tyrosine phosphorylation has been proposed as a marker to evaluate capacitation process. The classical approaches to study tyrosine phosphorylation have been through Western Blot, which does not provide information about changes at cellular level, and Immunocytochemistry, used to evaluate sperm specific sites of phosphorylation of a very limited number of cells (< 200). This work describes a new semi-automatized analysis of human sperm protein tyrosine phosphorylation during capacitation process using image-based flow cytometry. We have developed a segmentation strategy to measure cell specific sites of tyrosine phosphorylation in a relevant number of sperm cells (>2000), and identify sperm subpopulations with different protein tyrosine phosphorylation patterns: no phosphorylation, head or tail phosphorylated, and both head and tail phosphorylated.

We evaluated the level of tyrosine phosphorylation in sperms incubated in capacitation supporting media for 0, 4, 8, 18 and 24 hours, and found that tyrosine phosphorylation increases in a time-depending manner, as it has been reported by other techniques. Interestingly, this increase occurs only in the sperm tail but not in the head, consistently with that reported for other mammals. Additionally, sperm with both head and tail phosphorylated increased from 30 to 80% during capacitation time, whereas sperm with no phosphorylation and only head phosphorylated decreased from 30 to 10%, and sperm with only tail phosphorylated remain in the same proportion (~10%) during all the capacitation time. Surprisingly, the above mentioned has never been reported for human sperm. Recently it was reported that FER kinase is the enzyme responsible for the increase in protein tyrosine phosphorylation in mouse sperm. We test if this protein is also responsible of the increase in tyrosine phosphorylation in human sperm. Pharmacological inhibition of FER kinase produced a reduction of tyrosine phosphorylation, this decrease occurs mainly in the sperm tail, as it was expected considering the tail specific localization of this enzyme in mouse sperm. These results suggest the participation of this enzyme in human sperm capacitation process. Further work will be required to understand the relevance of this different sperm protein tyrosine phosphorylation patterns in male fertility.

**Key Words:** *Sperm capacitation, protein tyrosine phosphorylation, image-based flow cytometry, FER kinase*

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# *Moringa oleifera* leaf extract preserves mitochondrial respiratory activity in HepG2 cells exposed to hyperglycemia.

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Hyperglycemia observed during diabetes is well known to induce ROS generation by affecting mitochondrial integrity in several cell types. In our previous studies with liver of diabetic rats, we have observed than *Moringa oleifera* extract protects the formation of mitochondrial supercomplexes, as well as restoration of mitochondrial capacity and GSH levels. Hence, to determine its potential role in mitochondrial respiratory chain, we used HepG2 cells exposed to 50 mM glucose. Cells were treated with 3 different conditions: moringa extract at different concentration (50–500µg/mL), 50 mM glucose, and the combination of both. After incubation of 24 h, cells were evaluated in parameters such as viability, maximal respiratory capacity, ROS production, UCP2 activity, supercomplex levels and apoptosis rate. First, after incubation with moringa, cells did not show any adverse effect over the viability or ROS generation, in contrast, glucose incubation caused a rise in ROS levels but the viability was not affected. Apoptosis rate was slightly higher in cells exposed to glucose, while moringa showed no statistical difference against control cells. Regarding mitochondrial respiration, we used the seahorse XF24 to determine the maximal respiratory capacity of the mitochondria, observing that cells under hyperglycemic conditions tended to have lower respiratory capacity compared to control. This condition was partially reverted when moringa extract was applied to hyperglycemic cells. Furthermore, incubation with glucose revealed an increase in the proton leak of mitochondria that responded to genipin and TTNPB, suggesting an activation of uncoupling protein 2. This activity was modulated when moringa extract was used. Finally, electrophoresis of samples revealed higher levels of supercomplexes in the cells treated with moringa compared to control, these levels were even higher in hyperglycemic cells, correlating with our data in a rat model.

Our data demonstrates that glucose can induce a mitochondrial impairment, ROS generation and further apoptosis. On this matter, moringa extract is capable of protecting mitochondria from hyperglycemia, confirming its potential role in diabetes therapy.

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# Pharmacological and Functional Characterization of the Endogenous $LPA_{1/3}$ Receptors in U87-MG Cells

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Lysophosphatidic acid (LPA) is one of the so-called “bioactive lipids” that participates not only in cell metabolism, but also as an autacoid and local hormone. This compound is involved in a large number of physiological processes, modulating the function of many organs and systems, such as the gastrointestinal apparatus, the nervous, immune and urogenital systems, etc. As lipid mediator, it takes part in embryonic development and it is also involved in the pathogenesis of many diseases, like fibrosis, inflammation, cancer, among many others. Finally, at the cellular level, it modulates migration, chemotaxis, proliferation, survival and other important processes that are highly related to cell communication.

As many other hormones, LPA actions are mainly exerted through a complex family of transmembrane proteins that are called G protein-coupled receptors (GPCR’s). Currently, there are six GPCR’s known for LPA that are designated $LPA_{1–6}$. In this work, we studied the LPA receptors that are endogenously expressed in the cell line U87-MG, a human primary gliobastoma that has not been examined for this phospholipid. This research would allow us to elucidate some of the signaling pathways through which this compound is carrying out its effects in the nervous system.

Our results indicate that LPA was able to increase intracellular calcium concentration ($_i[Ca^{2+}]$) and ERK1/2 phosphorylation. These effects were partially achieved using a selective agonist for $LPA_1$ (OMPT) and totally and partially blocked by a selective antagonist for $LPA_{1/3}$ (Ki16425) and $LPA_1$ (AM095) respectively. Along with a real time RT-PCR assay, this suggests that $LPA_{1/3}$ receptors are endogenously expressed in these cells, although $LPA_1$ is particularly presented in higher proportion. On the other hand, pharmacological activation of protein kinase C by phorbol myristate acetate (PMA) resulted in a decrease of $_i[Ca^{2+}]$ when cells were challenged with LPA, nevertheless this effect was reverted by specific PKC-inhibitors, suggesting that these receptors are highly modulated by conventional PKC isoforms. Interestingly, PMA and AM095 induced a decrease in the basal levels of $_i[Ca^{2+}]$ in a dose-response manner. Finally, cell-size was totally affected by LPA and OMPT, while this effect was totally and partially blocked by Ki16425 and AM095 respectively.

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# The crosstalk between several posttranslational modifications controled the transcriptional activity of deltalactoferrin

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Deltalactoferrin (ΔLf) is a transcription factor with antitumor activities. Posttranslational modifications such as *O*-GlcNAcylation and phosphorylation, efficiently modulate its transcription factor activity and stability. Recently we first showed that ΔLf is modified by SUMO-1 and mapped the five SUMO sites. In a second time, we produced a series of mutants for which only one site was preserved and a null-mutant in which all five SUMO sites were invalidated. We showed that all lysine residues were SUMO acceptors and that K13, K308 and K379 were the main SUMO sites. We studied the impact of SUMOylation on ΔLf activity and showed that SUMOylation negatively regulated the transactivation function of ΔLf. In a next time we investigated the crosstalk between different posttranslational modifications. We showed that K379 which is either ubiquitinated or SUMOylated, is a pivotal site for the control of ΔLf stability. We also showed that SUMOylation competes with ubiquitination and protects ΔLf from proteosomal degradation by positively regulating its stability. We demonstrated that K13 is the main acetylation site and that favoring acetylation at K13 reduced SUMOylation and increased ΔLf transcriptional activity. Collectively, our results indicate that multi-SUMOylation occurs on ΔLf to repress its transcriptional activity. Reciprocal occupancy of K13 by either SUMO-1 or an acetyl group may contribute to the establishment of finely regulated mechanisms to control ΔLf transcriptional activity. Moreover, competition between SUMOylation and ubiquitination at K379 coordinately regulates the stability of ΔLf toward proteolysis.Therefore SUMOylation of ΔLf is a novel mechanism controlling both its activity and stability.

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# Inherent Conformational Flexibility of $F_1$-ATPase α-Subunit

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The core of $F_1$-ATPase consists of three catalytic (β) and three noncatalytic (α) subunits, forming a hexameric ring in alternating positions. A wealth of experimental and theoretical data has provided a detailed picture of the complex role played by catalytic subunits. Although major conformational changes have only been seen in β-subunits, it is clear that α-subunits have to respond to these changes in order to be able to transmit information during the rotary mechanism. However, the conformational behavior of α-subunits has not been explored in detail. Here, we have combined unbiased molecular dynamics (MD) simulations and calorimetrically measured thermodynamic signatures to investigate the conformational flexibility of isolated α-subunits, as a step toward deepening our understanding of its function inside the $\alpha_3\beta_3$ ring. The simulations indicate that the open-to-closed conformational transition of the α-subunit is essentially barrierless, which is ideal to accompany and transmit the movement of the catalytic subunits. Calorimetric measurements of the recombinant α-subunit indicate that the isolated subunit undergoes no significant conformational changes upon nucleotide binding. Simulations confirm that the nucleotide-free and nucleotide-bound subunits show average conformations similar to that observed in the $F_1$ crystal structure, but they reveal an increased conformational flexibility of the isolated α-subunit upon MgATP binding, which might explain the evolutionary conserved capacity of α-subunits to recognize nucleotides with considerable strength. Furthermore, we elucidate the different dependencies that α- and β-subunits show on Mg(II) for recognizing ATP.

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# Single-molecule measurements of kinesin dynamics and DNA-protein interactions

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Abstract

The development of new technologies has made it possible to observe and manipulate biological macromolecules at the individual level, giving rise to the field of single molecule biophysics. Optical tweezers is one such technology, consisting of a diffraction-limited focused laser beam that traps micron-sized dielectric objects (such as polystyrene beads or cells), allowing manipulation and measurement of the motions and forces developed by single biological molecules attached to the trapped object. We present two experimental studies using an optical tweezers instrument designed and built in our lab at IPICYT: the dynamics of the motor protein kinesin and the elastic properties of single DNA molecules. Conventional kinesin is an essential motor protein involved in intracellular transport, ferrying cargo along microtubules (MTs). We report the observation of single kinesin motors stepping on MTs and a comparison of how the processive run length of kinesin changes in one- vs two-motor per cargo configurations. In another experiment, we describe measurements of the contour length and persistence length (Lp) of single dsDNA molecules. Our measurement Lp = 47 nm is in excellent agreement with previous reports. Additionally, we are able to image the geometry of DNA-protein complexes using atomic force microscopy. These results constitute progress towards studying the dynamics of a single transcription factor binding to DNA at the individual level.

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# *In vivo* detection of compounds from plants by mass spectrometry using low-temperature plasma (LTP) ionization

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Mass spectrometry (MS) has become one of the most important tools for the analysis of biomolecules, such as natural products, peptides and proteins. For MS based analyses, the molecules need to be converted into ions. This process takes place in an ionization source. Novel ambient ionization sources permit the detection of compounds directly from samples with little or no sample pre-treatment.

Among these techniques, Low Temperature Plasma (LTP) ionization arises as a promising technique for *in vivo* analysis of compounds, because it allows the direct detection of biomolecules, whilst causing only mild damage on the studied organisms. There is no commercially available prototype, however recently a 3D printed device was developed in our research group [1].

The response of *Nicotiana tabacum* to mechanical damage is dependent on auxin production and involves a variety of regulatory networks that lead to accumulation of nicotine in the plant in stem and leaves. The metabolic characterization was performed using GC-MS [2], but a time-resolved chemical reaction on damage has not been reported. Using a 3-D printed LTP device we detected nicotine directly from *N. tabacum* plants, and monitored changes in the concentration of this metabolite following mechanical damage. Our findings demonstrate that LTP-MS offers a novel analytical approach towards the *in vivo* detection of biomolecules.

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# Hsp90α and Hsp90β are differentially expressed during the anti-proliferative and anti-apoptotic effect of the 17-DMAG inhibitor and thus dictate the treatment resistance of prostate cancer cell lines.

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***Introduction.*** Prostate cancer (PC) is the second most common form of cancer in men worldwide. Nowadays, there are several therapies under evaluation for the treatment of PC, such as the inhibitors of the heat shock protein 90 (Hsp90), they have been used as therapy for numerous carcinomas and they have exhibited promising results. Hsp90 inhibition is of particular significance for prostate cancer as Hsp90 is overexpressed in prostate cancer cells compared with normal prostate epithelium, which means, that the correct function of Hsp90 is essential for the growth and survival of tumor cells and thus provides a potential selective target. In this regard, Hsp90 of tumor cells has higher affinity for inhibitors as an indirect consequence of the activity of its co-chaperones. On the contrary, some Hsp90 inhibitors increase toxicity and resistance of cancer cells induced by poorly characterized mechanisms, and through the interaction of survival signals, that occurred as side effects of treatments. For this reason, we wanted to find out, whether the mechanism by which some types of PC become resistant to some Hsp90 inhibitors depends on changes in the expression of two of the main cytoplasmatic Hsp90 isoforms: Hsp90α and Hsp90β, since it has been reported that these isoforms have a pivotal role on the cancer development and progression. Interestingly, recent studies have suggested that some Hsp90 inhibitors could be selective of isoform and thereby each Hsp90 inhibitor should affect differently the expression and activity of the Hsp90 isoforms and thus their client proteins. ***Aim.*** To determine whether Hsp90α and Hsp90β are related with the development of resistance to the inhibition with 17-DMAG. ***Materials and Methods.*** Three prostate cell lines were used, from which two of them were prostate cancer cell lines (DU145 [PTEN +/-]) and PC-3 [PTEN -/-]), the third prostate cell line was a normal prostate epithelial cell line (PrEC). First, all the cell lines were treated for various periods of time with several concentrations of the Hsp90 inhibitor 17-DMAG (50ηM-1.0μM); then cell proliferation was measured with the MTT assay. From this experiment just a concentration (500nM) was chosen as well as two periods of time (18h y 72h) where a difference in cell proliferation among all the cell lines were found. After that, in order to find out in which phase of the cell cycle the cells were, the cellular DNA was dyed with propiduim iodide (PI) and the samples were read on a flow cytometer. To validate the results obtained with PI and to see the nucleous integrity, the cells were stained with DAPI. At the same time, several Western Blot assays were done for Hsp90α and Hsp90β, including as client proteins involved in biological processes evaluated to: p-mTOR, HIF-1α, Hsp70, Hsp27, Bcl-2, BAX, caspase 3, cleaved caspase 3, PARP, Akt, p-Akt) in order to see the differences in protein expression. In vitro scratch assay was done to measure cell migration just in the groups we have selected previously. ***Results.*** As it was expected, differences among all the groups were found in terms of protein expression, proliferation, migration, DNA integrity and cell cycle, but interestingly all the evidence indicate that DU145 seems to be a resistant prostate cancer cell line to 17-DMAG inhibitor, even more resistant than the PrEC cell line. On the other hand, PC-3 is a sensible cell line to 17-DMAG. Furthermore, the resistance of DU145 cell line, seems to be related to the diminished expression of the Hsp90α isoform. On the contrary, the Hsp90β isoform doesn't seem to be related to the cell resistance. This highlights the need of the properly characterization of the different subtypes of prostate cancer with the sole aim of choosing the adequate drug therapy for enhancing the disease outcome.

# Light chain amyloidosis: from immunoglobulin unfolding to amyloid-like protofibrils.

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Light chain amyloidosis (AL) is a misfolding disease characterized by the extracellular deposition of immunoglobulin light chains (LCs) as insoluble aggregates [1]. Among the LC families, lambda 6a is highly frequent in AL patients [1]. Its germline protein (6aJL2) and point mutants (R24G and P7S) are good models to study fibrilllogenesis, because of their stability and fibril formation characteristics [2,3]. In addition, acidic pH and the presence of residual secondary structure favor fibril formation in other members of this family [4]. Although many of the clinical aspects of this pathology are known, the molecular mechanism of aggregation remains unclear. The conformational changes resulting in LC intermediates capable to form fibrils have not been characterized at the atomic level. At the very least, they should involve eliminating the anti-aggregation motifs [5] and rotating the β sheets so the β strands become parallel [6]. In order to gain understanding of the effect of mutations and low pH on the dynamics and unfolding of these proteins, we have performed molecular dynamics simulations at neutral and low pH, and at increasing temperatures (298, 398, 448 and 498K). This approach allows us to sample the conformational landscape, to find intermediates able to form fibrils. We found that mutations and low pH compromise the stability of the anti-aggregation motifs leaving the edges of the β sandwich unprotected, each by a different mechanism, though. Point mutants modify the contact networks and hydrogen bond patterns surrounding the mutation and extend across the protein, affecting the CDR1 and the C'-C'' protective loop. At low pH, the most noticeable effect is the destabilization of the loop connecting strands E-F, close to C-terminus of the protein, allowing water access to the hydrophobic core. From high temperature simulations, we identified unfolding intermediates that are close to the native conformation while having the sides of the β sandwich denatured. We quenched these at 298K and they held their structured β core during 0.5 μs of simulation time. One of the intermediates of the 6aJL2 protein was stable enough to be extended into a dodecameric protofibril model with two isomeric forms. Both protofibril models were simulated for 1 μs, allowing their structural and dynamic characterization. We found molecular hallmarks that lead to the amyloidogenic pathway in these proteins. Also, this is the first time that a protofibril model that would lead to the correct cross-beta diffraction pattern is proposed for this family of proteins.

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# Phylogenetic profile, sequence conservation and genetic complementation assays of the protein family EFL1

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During the ribosome biogenesis in eukaryotes, the 60S and 40S ribosomal pre-subunits are exported to the cytoplasm to undergo the last maturation steps. In *Saccharomyces cerevisiae*, the 60S subunit arrives in the cytoplasm loaded with Tif6, a protein which prevents the premature association of the 60S and 40S subunits. The GTPase Efl1 and Sdo1, its guanine exchange factor, catalyse Tif6 release from the 60S subunit and trigger 80S particle assemble. It has been observed that Tif6 and Sdo1 are conserved in the Archaea and Eukarya domains, whereas EFL1 members have only been found in yeast and mammalian.

In order to study the evolutionary conservation of the EFL1 protein family, we built a phylogenetic profile through identification and retrieval of its different members. This study showed that the EFL1 proteins are highly conserved and are exclusive of the Eukarya domain. On the other hand, a multiple sequence alignment showed that these proteins preserve important functional regions and most likely they share the same tridimensional fold. Furthermore, the phylogenetic analysis showed that the EFL1 family shares a common origin with EF-2 and EF-G proteins. Interestingly, the cladogram suggests that EFL1 and eEF-2 where generated after a gene duplication phenomenon; while the latter maintained its function in protein synthesis the other one became specialized in ribosome biogenesis.

Finally, to evaluate a conserved function among the EFL1 members we carried out genetic complementation assays in *S. cerevisiae efl1Δ* cells using the EFL1 orthologue of *Mus musculus*, *Homo sapiens*, *Schizosaccharomyces pombe* and the *Archaeoglobus fulgidus* aEF-2. The results showed that none of the evaluated members were able to complete the *EFL1* gene function in *S. cerevisiae*. Similar studies with SBDS family members showed null functional conservation among them, suggesting a coevolution phenomenon between EFL1 and SBDS protein families. To demonstrate this, we assayed the double genetic complementation using both family proteins. The orthogonal pairs evaluated neither complemented the function of the yeast proteins. However, our results suggest that the recognition is specie-specific and requires not only that between the SBDS and EFL1 proteins, but also the recognition with 60S ribosomal subunit. Together this suggests that the coevolution phenomena occurred not only amongst the accessory proteins, the EFL1·SBDS complex but with also on its interaction surface in the ribosomal subunit.

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# Matrix metalloproteinase 13 (MMP13 or collagenase 3) play a key role during resolution of lung fibrosis.

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Disturbances in macrophage function can lead to aberrant repair, such that uncontrolled production of inflammatory mediators and growth factors, deficient generation of anti-inflammatory macrophages, or failed communication between macrophages and epithelial cells, endothelial cells, fibroblasts, and stem or tissue progenitor cells all contribute to a state of persistent injury, and this could lead to the development of pathological fibrosis. Extracellular matrix remodeling, including collagenolysis, is mediated mainly by matrix metalloproteases (MMPs), such as MMP-1, MMP-13 and MMP-8. We have use a mutant mouse containing loss-of-function deletions in Mmp13 gene as a deficient degradative system model to evaluate the role of these collagenase 3 in extracellular matrix remodeling after lung injury. Mmp13 knockout mice were treated with bleomycin and the inflammatory and fibrotic response were analysed at 7, 21, 28, 35, 42, 49 and 56 days post-treatment. Collagen lung content was assessed measuring hydroxyproline. Stained lung sections were scored blindly for severity and extent of the lesions and percentage of fibrosis. MMP-2 and MMP-9 levels were examined in lungs and bronchioalveolar lavage fluid by gelatin zymography. Additionally, mRNA expression level of Mmp1a, Mmp2, Mmp7, Mmp8, Mmp9, Mmp13, Mmp14, Timp1 and Timp3 was evaluated by real time PCR. We observed that both, WT and Mmp13 knockout mice experienced inflammatory cell infiltration at 7 days after bleomycin. However, inflammatory response in the lungs from Mmp13 knockout was characterized by enhanced neutrophilic infiltration compared to WT mice. Likewise, Mmp13 deficient mice exhibited more extensive, dense and severe fibrosis characterized by increased lung collagen deposition. It has been suggested that MMP2 and MMP9 may also have collagenolytic activity under physiologic conditions. In this context, we evaluate MMP2 and MMP9 activity in lungs and bronchio-alveolar lavage fluid from WT and Mmp13 knockout mice, in order to analyse whether MMP2 or MMP9 could at least partially compensate for the absence of MMP8 and MMP13 enzymes. We found that the level of both enzymes were significantly higher in both, bronchio-alveolar lavage and lung from Mmp13 deficient mice compared with WT bleomycin-treated mice at 28 days of treatment. These data could suggest a partial compensation of MMP2 and MMP9 for the absence of MMP8 and MMP13 enzymes in fibrotic lung. However, we observed a delay in fibrosis resolution in the lungs from Mmp13 deficient mice, indicating a very specific role for these collagenase during extracellular matrix degradation after injury and fibrosis. Additionally, we found an increase in M2 macrophages in lung from Mmp13 deficient mice and higher level of several pro-inflammatory and pro-fibrotic cytokines. Our findings indicate that the absence of both MMP8 and MMP13 increases the severity of bleomycin-induced pulmonary fibrosis, suggesting also a protective role for these proteases. We propose that the Mmp13 knockout mice represent a novel in vivo model to elucidate the functional relevance of collagenase 3 in fibrotic lung disorders and fibrosis resolution.

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# Identification of membrane proteins of *Helicobacter pylori,* which bind human haemoglobin

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*Helicobacter pylori* is a pathogen that causes peptic ulcer and gastritis. This bacterium survives in several environments into the human host. *H. pylori* scavages iron from human haemoglobin or haem using membrane proteins. Nonetheless the identity of these proteins remains unknown, therefore the mechanism of iron acquisition HpGroEL is not clear. We previously have identified by *in silico* analysis three proteins of *H. pylori* related to haemoglobin-binding (Hb-binding), two of them (FrpB1 and FrpB2) bound Hb and supported the cellular growth of *Escherichia coli* when Hb was supplied as only iron source. In addition of these results, *H. plylori* expresses proteins which could be involved in Hb-binding, however their identity remains undeterminated. This result encoreges us to investigate more about Hb-binding mechanism in this pathogen. Membrane proteins were extracted and purified by haem affinity chromatography. Eigth proteins were identified by mass spectrometry. In this set of proteins FrpB3 was identified. This strategy allowed us to complete the family of FrpB proteins of *H. pylori*. All the proteins identified in this work suggest that this pathogen has a enormous battery of Hb-bindig proteins which are necessary perhaps to invade several tissues present in the human host.

# SMc03960 is a thioesterase from *Sinorhizobium meliloti* with broad substrate specificity capable of producing 2-tridecanone.

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A *fadD* mutant of *Sinorhizobium meliloti* shows swarming motility and accumulation of free fatty acids during the stationary phase of growth. Recently, the methyl ketone 2-tridecanone (2-TDC) was detected and associated with the swarming phenotype of the *fadD* mutant, suggesting a possible function of 2-TDC as a signal molecule. Methyl ketone synthase 2 (MKS2) has been reported as a thioesterase responsible for the production of 2-TDC in wild tomato. Nevertheless, *in bacteria little* is *known* about the biosynthesis and function of 2-TDC.

SMc03960 is a conserved hypothetical protein of *S. meliloti* with homology to MKS2. Heterologous expression of SMc03960 in *Escherichia coli* led to the formation of significant amounts of the methyl ketones 2-TDC and 2-pentadecanone. Furthermore, expression of SMc03960 in *E. coli* caused accumulation of free fatty acids in cells as well as in the culture medium. Thioesterase activity of purified His-SMc03960 was assayed against a wide range of acyl groups linked either to acyl carrier protein (ACP) or to coenzyme A (CoA). Among the substrates tested, myristoyl-CoA and myristoyl-ACP were the best substrates with apparent Km values of 19 µM and 10 µM, respectively. MKS2 uses the fatty acid biosynthesis intermediary 3-oxo-acyl-ACP as substrate to produce 2-TDC. Through a series of sequential reactions with purified enzymes and employing ACP, malonyl-CoA and lauric acid, 3-oxo-myristoyl-ACP was synthesized. His-SMc03960 hydrolyzed 3-oxo-myristoyl-ACP leading to the formation of 2-TDC *in vitro*.

In summary, in this work we demonstrate *in vivo* and *in vitro* formation of 2-TDC by means of SMc03960. Furthermore, SMc03960 shows broad functional diversity since it can hydrolyze acyl-CoAs and acyl-ACPs. SMc03960 is an ortholog of the Tol-Pal system-associated thioesterase YbgC, whose function is unknown. Therefore, we hope to unravel the role of *YbgC-like thioesterases with our studies.*

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# Expression profile of Hsp90 alpha and Hsp90 beta identifies a patients subgroup with renal cell carcinoma with lower survival

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**Background:** Clear cell renal cell carcinoma (ccRCC) is the most frequently type of renal cancer (RC), affecting adults primarily, being more frequent on men (2:1 regarding women). GLOBOCAN database reported in the year 2012 that RC in Mexico took the 8° place of incidence in the male population. The first choice treatment for RC patients is the surgical (nephrectomy), having the peculiarity of being resistant to radiotherapy, chemotherapy and immunotherapy. Therefore, the development of drugs designed to inhibit signaling pathways that stimulate angiogenesis and proliferation of RC offers new hope in clinical therapy. The current therapeutic against various types of cancer have recognized inhibitors of Hsp90 protein as a promising mechanism on cancer fighting. On this field, Hsp90 is defined as an essential chaperone-protein for stability and activation of diverse oncogenic proteins, among which include: kinases (e.g. Akt, mTOR) and transcription factors (e.g. HIF-1 alpha). In a wide variety of tumors it has been identified that Hsp90 is overexpressed 2-10 times, correlating with poor prognosis. Preliminary results show that principal isoforms of Hsp90, Hsp90 alpha (Hsp90a) and HSP90 beta (Hsp90b), are differentially expressed on a variety of cancer cell lines. In 2010, we reported that Hsp90a and Hsp90b differentially regulate the activity of kinase B/Akt; however, the role of Hsp90 approach for the therapy of renal cell carcinoma is unknown, and even less the specific role of each isoform. **Aim:** Obtain the expression profile of Hsp90 alpha and Hsp90 beta proteins on kidney tissue from patients with ccRCC and determine its possible role as molecular marker in progression, studying Akt/mTOR/HIF-1a proliferation pathway as main effector signaling pathway in the development and progression of ccRCC. **Material and Methods:** For the prospective study, twenty renal histological sections were included from patients diagnosed and classified with ccRCC. In them, the expression level of Hsp90a and Hsp90b proteins were determined by Western blot (WB) and immunohistochemistry (IHC) assays, as well as their effector proteins involved in the proliferation pathway Akt/mTOR/HIF-1a. **Results:** The expression profile between Hsp90a and Hsp90b acquired by Western Blot and Immunohistochemistry identifies a patients subgroup with ccRCC in a metastatic clinical stage and lower survival, considering 24 months post-nephrectomy. The expression pattern between Hsp90a and Hsp90b has direct association over expression and activation profile of effector proteins involved in the Akt/mTOR/HIF-1a proliferation pathway. **Conclusion:** The expression pattern between Hsp90a and Hsp90b on renal tumor tissue versus normal renal tissue is able to identify and group patients with ccRCC in metastatic clinical stage and with a worse prognosis assessed by overall survival, associated with activation of Hsp90 client proteins involved in Akt/mTOR/HIF-1a signaling pathway.

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# Identification of druggable binding pockets in the molecular surface of the TATA binding protein from eukaryotic parasites.

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The basal transcription machinery is conserved across eukaryotes. One of these central proteins is the TATA binding protein (TBP), which recognizes the TATA-box DNA sequence and functions as a structural scaffold to initiate transcription by the three RNA polymerases [1]. Proposing this protein as a potential druggable target for treatment of parasitic infections follows these considerations: 1) all organisms carry out transcription, so it is lethal for the parasites to inhibit this process; 2) despite the conservation of TBP, parasitic TBPs show more divergent sequences with respect to human TBP, which suggests surface differences [2]; 3) structural and biochemical information of TBP with other proteins is available [3], allowing us to propose function-based drug-binding sites and also to expand the target set. To analyze this protein as a druggable target, we selected a set of parasitic and vector TBPs, predicted structural models, and carried out molecular dynamics simulations for these TBPs, to generate structural ensembles for virtual screening and to study their structural differences. The final structural ensembles were selected based on 2D-RMSD clustering and local collections of side chains for each binding pocket, which were predicted for representative structures obtained from clustering. The predicted pockets and electrostatic potential were calculated to identify possible binding pockets. As an initial approximation we evaluated the binding of FDA-approved drugs in ZINC to the selected structural ensembles. We used the Autodock Vina program [4] for docking, with rigid receptors and flexible ligands, saving five poses for each ligand. According to the pairwise sequence analysis of each parasitic/vector TBP with respect to human TBP, we found that parasitic/vector TBPs showed variable residues at specific sites of the surface, some of which correspond to residues of the predicted pockets. The initial docking analysis shows that the interaction affinity of FDA-approved drugs is similar in pockets shared between parasite and human TBP, but we found more selectivity for pockets present only in parasitic TBPs. Ligand selectivity to parasite TBPs were greater than 1.5 Kcal/mol and binding energies corresponded to Kd's in the order of micro to nanomolar, indicating potential ligands for more exhaustive analysis.

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# Impact of presumed CD49f antagonist in the stemness of breast cancer cells

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Breast cancer is the second cause of cancer death in women(a) and most of those deaths are due to drug resistance and or cancer recurrence(b). The cancer stem cell model suggests that stem-like cancer cells are responsible of resistance and recurrence in cancer patients(c) making them excellent targets for anti-cancer therapies. One of the cell surface proteins implied in the stemness of breast cancer cells is the alpha integrin CD49f. Knock-down of this protein impairs the stemness of breast cancer cells both, *in vivo* and *in vitro*(d).
Aiming to target breast cancer stem cells, we selected potential CD49f antagonists from a collection of FDA-approved drugs by structure-based virtual screening. Our data reveled that several of the identified compounds decrease with different potencies the capacity of CD49f+ breast cancer cells to bind the ligand laminin, suggesting that the selected compounds antagonize the receptor. Additionally, functional studies showed that some of these compounds limit the clonogenic capacity of CD49f+ breast cancer cells *in vitro* without affecting whole population cell viability. At the moment, we are analyzing the inhibition of CD49f-activated pathways in presence of the selected antagonists and the capacity of these compounds to affects *in vivo* tumorigenicity.
Thus, we have identified some drugs that could be useful to target breast cancer stem cells. Since the pharmacokinetics and toxicology of these drugs is known, they could *easily* be ready for clinical trials. We demonstrated that the *in silico* strategy employed can successfully identify antagonists of receptors with relevant roles in breast cancer stem cells.
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# Glucose regulates lifespan through a network of stress-responsive transcription factors in *Caenorhabditis elegans*.

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Glucotoxicity refers to the deleterious effects of chronic hyperglycaemia that promotes toxicity, and is considered as a contributing factor to develop obesity, diabetes, cardiovascular diseases, and as a determinant of reduced lifespan and aging. To study the molecular mechanisms involved in glucotoxicity, we are using the worm nematode *Caenorhabditis elegans* as a model, as the metabolism of carbohydrates and lipids is similar to that of mammals, and 18 signaling pathways, including the insulin pathway, are homologous to that of humans. Its genome contains over 19,000 protein-coding genes, of which 60% are conserved with humans. Finally, it can be fed with high-carbohydrate diets.

We established a model of glucotoxicity by feeding the worm with increasing amounts of glucose (from 0 to 100 mM glucose added to the growth medium) that resulted in the internal accumulation of this sugar, and caused an increased triglyceride´s content. Interestingly, the lifespan of glucose-fed worms was reduced inversely to the amount of glucose fed.

As a way of identifying the transcription factors that are mediating the lifespan´s reduction, we analyzed the mRNA´s accumulation of genes that code for several transcription factors related to stress-responsive pathways, such as SBP-1/SREBP, CEP-1/p53, SKN-1/NRF-2, DAF-16/FoxO, CRH-1/CREB and HIF-1/HIF-1a. We found that the expression of *cep-1*, *crh-1* and *sbp-1* was up-regulated, while that of *daf-16* and *skn-1* was down-regulated in
worms grown at glucose 100 mM. In contrast the expression of *hif-1* did not change. In addition, we noted that toxicity and mRNA´s accumulation changes were maximal at glucose 100 mM.

To evaluate the participation of these transcription factors in lifespan regulation during glucotoxicity, we silenced the expression of *sbp-1*, *cep-1*, *crh-1* and *hif-1* by RNAi, while studying *daf-16* and *skn-1* participation with mutant strains. Surprisingly, we found that silencing of *cep-1*, *hif-1* and *crh-1* increased lifespan at 100 mM glucose, with respect to the control. In contrast, lifespan was decreased in *sbp-1(RNAi)*, and in the *daf-16* and *skn-1* mutant strains, with respect to the control, in worms grown at 100 mM glucose. We are now trying to better understand how these transcription factors determine lifespan in glucose-fed worms.

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# Microarray Analysis of differentially expressed genes in trophoblast with iodine deficiency

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## Abstract

Epidemiological evidence shows the importance of iodine intake during pregnancy, associating its deficiency with preeclampsia. The molecular mechanism responsible has not been described, although it has been reported that iodine deficiency affects trophoblastic differentiation and migration.
In order to determine the functional role of iodine in gestation we analyzed the effect of iodine deficiency induced with 1 mM perchlorate on gene expression in human trophoblasts BeWo, using microarrays (IFC-UNAM) 34,597 genes data base. Microarray analysis identified 48 transcripts up-regulated with a z-score> 2, and 112 down-regulated transcripts with a z-score> 2 in iodine deficiency trophoblast. Regulated genes are involved primarily in cell cycle, proliferation, apoptosis, chromatin organization, response to stress, fat metabolism, ion transport, biochemical pathways, hormonal function, adhesion, invasion and cell migration, among other functions. The RT-PCR real-time analysis confirmed the expression of genes involved in invasion, migration, and cell adhesion (GAST7, NSD3L, plakophilin 2 Emilin, Dynatic 3 protocadherins 11, 15 and gamma A12); cellular proliferation genes (EGFR SAFB1, and ACE2); genes associated with oxidative stress (Annexin A4, HIF); as well as transcription factors and fat metabolism genes (Apoliprotein E, SREBF1, and C/EBP-beta). In addition, iodine deficiency induces ROS formation and translocation to the nucleus HIF1-alpha and increased protein of Snail and decrease C/EBP-beta, by western blot.
This study shows for first time that iodine deficiency increases levels of ROS, altering gene expression in human trophoblast BeWo. Moreover it induces translocation of HIF-1 alpha, inducing expression of Snail. In this study iodine deficiency decreases the expression of C/EBP-beta to RNA and protein level. Indicating that adequate levels of iodine contribute in normal physiology of trophoblast cells. This study had financial support from CONACyT grant no. CB-2012-01-176513 and Garduño Gabriel was supported by a graduate fellowship from CONACYT (24076).

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# Co-regulation of *CSD1 and ADH1* mRNAs by miR398 and miR2119 in response to stress in *Phaseolus vulgaris*

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Plant microRNAs are commonly encoded in a transcript containing a single precursor microRNA. Processing by DICER-LIKE 1 and associated factors results in the production of a single microRNA:microRNA* duplex of 20-24 nucleotides in length, followed by selection of one strand of the duplex and its incorporation into an AGO-containing silencing complex to direct silencing of a mRNA containing a complementary target sequence.

Commonly, individual microRNAs are encoded by a single *MIR* locus. Particular *MIR* loci have been described to contain more than one precursor stem-loop structure, thus encoding more than one microRNA in the same transcript, such as the *MIR*395 family, encoding up to eight precursors in a single transcript in rice. Here we describe a unique case where the evolutionarily-conserved miR398 is encoded in the same transcript as the legume-specific miR2119. The bicistronic arrangement found in *Phaseolus vulgaris* (common bean) is also conserved in other legumes. In *Phaseolus vulgaris* mature miR398 and miR2119 are downregulated in response to drought as revealed by Northern blot and RT-qPCR experiments. We show that they are both functional as they target the *COPPER SUPEROXIDE DISMUTASE 1 (CSD1)* and *ALCOHOL DEHYDROGENASE 1 (ADH1)* mRNAs for cleavage, respectively, as demonstrated by 5'RACE assays. Accordingly, upon drought conditions the accumulation of *CSD1* and *ADH1* mRNAs increased. Moreover, when we overexpressed the bicistronic precursor using the transgenic hairy root system, mature microRNAs were highly accumulated and consistently, both target mRNAs showed reduced accumulation levels. Our results indicate that the joint down-regulation of miR398 and miR2119 is utilized to allow the accumulation of the mRNAs encoding *CSD1* and *ADH1* in response to drought in common bean and other legumes as well, suggesting that reactive oxygen species and fermentation metabolism are closely coordinated under drought conditions, and possibly other forms of stress as well.

This project is supported by funds from CONACYT (151571) and DGAPA-PAPIIT (IN-205015) granted to JLR.

# Novel mechanisms of lifespan extension revealed by genome-wide screening of dietary-restriction factors in yeast

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The limitation of calories or other specific of nutrients in the diet, also known as dietary restriction (DR), is the only non-genetic intervention known to extend the lifespan of a wide range of model organisms from yeast to mammals. The genetic analysis of chronological lifespan (CLS) in the budding yeast *Saccharomyces cerevisiae* has been pivotal in unraveling some of the mechanistic bases of lifespan extension by DR; however, comprehensive genome-wide information is still missing. Here, we used a novel large-scale assay to measure the CLS of a collection of ~4,000 knockout mutants aged under both nutrient-rich or dietary-restricted media. To this end, yeast CLS was measured by monitoring the relative survivorship of mutant and WT-reference strains labeled with different fluorescent proteins. Such high-resolution output allowed us to quantitatively determine the effect of each single-gene knockout on CLS and the interaction of this phenotype with DR. Our results revealed over 400 genes involved in the process of lifespan extension. Functional classification of such DR factors exposed the biological processes implicated in this response, many of which had been previously characterized in the context of lifespan-regulation, such as autophagy and mitochondrial function. Interestingly, we found that a major portion of DR factors are regulated by Ste12 transcription factor, which its role in aging had not been described. We show that Ste12 is necessary for lifespan extension in response to DR and unraveled a link of this transcription factor to the TOR signaling pathway. Our comprehensive functional-genomics approach provides new insights into the cellular mechanisms of lifespan extension by dietary restriction.

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# Genetic and genomic analysis of zygotic genome activation in *Arabidopsis*

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Fertilization produces a diploid zygote from two distinct haploid genomes, which then function together to direct embryo development. The beginning of large-scale zygotic transcription is referred to as zygotic genome activation (ZGA) (reviewed in Del Toro-De León et al., *COPB*, 2016). Using a functional analysis of early paternal gene activity, we have recently provided an explanation for a long-standing controversy regarding ZGA in plants (Del Toro-De León et al., *Nature*, 2014). We used embryo defective mutants to evaluate the ability of wild-type paternal alleles to complement phenotypes conferred by maternally inherited mutant alleles. Our experiments showed that in isogenic embryos, most paternal alleles are not fully functional immediately after fertilization, suggesting that the maternal genome makes the dominant contribution to early stages of development. We also demonstrated that hybrid genetic backgrounds have a significant effect on paternal allele activation.

Using a Col x Tsu hybrid transcriptome, we found an overwhelming bias toward maternal transcripts in the first 2 days of embryogenesis, with paternal contributions steadily increasing until the heart stage, when parental contributions are approximately equivalent. We hypothesize that these parent-of-origin effects on transcription in early embryos are the result of two processes: a) transcriptional activation via specific *cis* regulatory motifs (as well as *trans* acting factors), and b) silencing of paternal alleles due to epigenetic marks established in the gametes. We are currently using a YFP/RFP reporter line system analysis to simultaneously assess maternal and paternal allele expression during embryogenesis. In addition, we have identified three motifs that are highly enriched in early expressed genes. Current work is focused on evaluating the importance of the identified motifs in promoting transcription in early embryos, as well as determining the relative gametophytic and zygotic contributions to maternal transcript dominance in early embryos.

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# Local DNA topology is a selection factor that determines the association of DNA to the nuclear matrix.

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In metazoan cells during the interphase nuclear DNA is organized in supercoiled loops anchored to a proteinaceous compartment known as the nuclear matrix (NM), obtained after extracting the nucleus with non ionic detergent and high-salt. DNA sequences of variable length known as matrix attachment or addressed regions (MARs) mediate the anchoring to the NM. In mammals there is no consensus sequence or clear-cut properties shared by MARs and so, for decades the molecular basis of the interaction between DNA and the NM has been an unsolved conundrum. MARs are operationally classified in structural, that resist extraction with high salt, and facultative, that do not resist such an extraction. The former are known as LARs and define structural DNA loops anchored to the NM. Using an experimental approach based on topological principles we have previously determined the organization into structural DNA loops of the genomic region of the albumin family in primary rat hepatocytes. Our method allows us to infer which parts of such a region are or contain the LARs. In the present work we first demonstrated that the DNA sub-regions ~ 2Kb in length, corresponding to or containing the inferred LARs, are bound in situ to the NM of hepatocytes. Next we tested the ~ 2Kb LAR-containing sequences for their ability to bind to hepatocyte-NM proteins in vitro under stringent conditions. Surprisingly the results were negative. However, short sequences ($\leq$ 300 bp) belonging to the previously tested ~ 2Kb sequences bind with high-affinity to the NM proteins. Moreover, we found that any sequence from the genomic region under study binds with high-affinity to the NM-proteins provided that is $\leq$ 300 bp in length, no matter if such a sequence is actually located in situ at the tip of a DNA loop instead of bound to the NM. Current biophysical evidence indicates that chromosomal DNA dissipates structural stress by looping and supercoiling along its axis. Binding of the spontaneously forming loops to the NM stabilizes the chromosomal DNA and preserves its integrity. DNA is one of the stiffest natural polymers but fragments significantly longer than its average Kuhn length (estimated at 300 bp for DNA) spontaneously deform and may adopt complex topologies in suspension at physiological temperature. Our results suggest that NM proteins have intrinsic affinity for any DNA whose local topology is closer to the straight line. Therefore a complex 3D topology is a negative selection factor that impairs the binding of DNA to the NM. Spontaneous looping and supercoiling of DNA is driven by entropic forces. This coupled to the intrinsic affinity of NM-proteins for DNA with simple-topology determines a self-organizing system that results in a robust nuclear higher-order structure defined by the set of DNA-NM interactions.

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# Npa3/*Sc*Gpn1 carboxy-terminal domain is dispensable for cell viability and RNA polymerase II nuclear targeting but critical for microtubule stability and function

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The biogenesis of RNA polymerase II (RNAPII) depends on Gpn1 in yeast and human cells. In human cells Gpn1 also mediates nuclear export of the RNAPII CTD phosphatase RPAP2. However, the exact molecular function of Gpn still unknown. Gpn1 is an essential protein in all eukaryotic cells examined. In Archaea the Gpn protein comprises only a GTPase domain while eukaryotic Gpn1 organizes as two independent modules: an N-terminal GTPase core and an acidic C-terminal extension. We hypothesized that in eukaryotic organisms the newly acquired C-terminal extension confers regulatory and/or additional properties to its essential GTPase core. To investigate the functional importance of the C-terminal extension in *Sc*Gpn1, we generated a *Saccharomyces cerevisiae* strain expressing only a C-terminal truncated version of *Sc*Gpn1 (*gpn1ΔC*). This *gpn1ΔC* strain was viable and proliferated normally, but the cells showed a marked increase in cell size. Nuclear localization of GFP-tagged RNAPII subunits Rpb1, Rpb2 and Rpb3 was unaffected in *gpn1ΔC* cells but they displayed a higher nuclear fraction of the phosphatase GFP-Rtr1, the human ortholog of RPAP2, suggesting a critical role for Gpn1 C-terminal extension in Rtr1 nuclear export. However, the most remarkable phenotype of *gpn1ΔC* cells was a decrease in microtubule stability and function, which was visible by a higher percentage of cells in G2/M, a delay in mitotic exit, and by an increase in sensitivity to the microtubule destabilizing agent benomyl. At a sublethal concentration of benomyl microtubule integrity was totally lost in *gpn1ΔC* but not in *Sc*Gpn1 expressing cells, pointing to a role for *Sc*Gpn1 in microtubule dynamics. Thus, we have discovered some *Sc*Gpn1 cellular functions exclusively carried out by the C-terminal extension and uncoupled these functions from RNAPII nuclear localization.

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# The CDK8 module of Mediator controls vegetative phase change in *Arabidopsis thaliana*

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The transition from the juvenile to the adult vegetative phase is regulated by a genetic pathway involving the microRNAs miR156 and miR172, and the miR156-targeted *SPL* transcription factors. Despite the extended knowledge about this pathway, very little is known about what it regulates these microRNAs. It has been recently reported that sugar promotes vegetative phase change by repressing miR156 (Yang et al., Yu et al., *eLife*, 2013). In our laboratory we have demonstrated that *CCT/MED12* and *GCT/MED13,* members of the CDK8 module of Mediator, also act upstream of the miR156-*SPL*-miR172 pathway (Gillmor et al., *Development*, 2014; reviewed in Buendía-Monreal & Gillmor, *Dev Biol,* 2016). One important question is whether the CDK8 module and sugar regulate miR156 separately or together. We have recently found that sugar and the CDK8 module regulate miR156 levels independently, as double mutants of *ch1* (a photosynthetic gene) and CDK8 module subunits show an additive effect on heteroblasty traits as well as in the regulation of miR156; furthermore, sugar treatment repressed miR156 expression in a CDK8 module-independent manner.

We are interested in exploring further whether other genes of the CDK8 module of Mediator regulate vegetative phase change, and in determining which specific genes of the miR156-SPL-miR172 pathway are under CDK8 module control. Our results show that *hen3/cdk8* mutants also exhibit a delay in flowering time and specific changes in heteroblasty; in addition, *hen3 cct* double mutants have an additive effect in delaying flowering time, indicating that *HEN3/CDK8* also plays an important role in the regulation of the reproductive transition. Expression analysis has revealed that only certain *MIR156* genes and their *SPL* targets are transcriptionally regulated by the CDK8 module. We are currently carrying out experiments to further characterize this spatio-temporal regulation, and we are going to use ChIP to determine which genes of the miR156-SPL-miR172 pathway are direct transcriptional targets of CDK8 module genes.

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# Identification of physiological and molecular responses-associated to abscisic acid under hydric stress in a new model of plant *Marchantia polymorpha*

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Water limitation is one of the most restricting factors for plant growth and reproduction, consequently affecting crop yields. To understand the genetic and evolutionary basis of plant responses to this environmental adverse condition, in this work we characterized the physiological, cellular and molecular responses to water deficit conditions in the basal liverwort *M. polymorpha.* This bryophyte is a representative of the most ancient plant lineages that colonized land, hence a relevant model to study the evolution of the plant responses to environments with water availability limitations.

We evaluated the development of thalli grown *in vitro* under optimal and stressful conditions using a controlled environment where we applied drought stress or by including different NaCl concentrations in the medium. Thalli exposed to drought or NaCl showed a decreased rate of growth and morphological anomalies. Also, stress treatments led to relocation of cell organelles; mostly due to changes in volume of the central vacuole, which correlated with adjustments of osmotic potential in plant tissues. Because Abscisic acid (ABA) is a phytohormone that has been shown to mediate plant responses to environmental stress, we also determined endogenous ABA levels. Consistently, we observed an increase in ABA upon NaCl or drought treatments, which correlated with the expression of a number of stress-related genes including *LATE EMBRYOGENESIS ABUNDANT* (*LEA*) genes, which are thought to protect cells from damage caused by water deficit. In this way, we identified *MpoLEA3* encoding for a group 3 LEA proteins, and *MpoLEA2* that encodes for a dehydrin or group 2 LEA member. We also determined mRNA levels of *MpABI1*, an ortholog of *Arabidopsis ABSCISIC ACID INSENSITIVE1* (*ABI1*), coding for a protein phosphatase involved in the ABA signaling pathway. Preliminary results indicate that in thalli exposed to drought, NaCl or ABA, there is an increase in transcript accumulation of *MpoLEA3* and *MpoLEA2*, while the levels of *MpABI1* mRNA do not change. These results suggest that stress signaling induced accumulation of *LEA3* and *LEA2* genes in order to protect cells from damage, while no changes in *ABI1* mRNA suggest that increases in endogenous ABA are processed by ABI1 at the protein level as has been documented in vascular plants.

The results in this work show the effect of water deficit in *M. polymorpha* at the physiological and cellular levels, and indicate that at least some responses to water limitation in this basal plant are mediated by ABA, which seems to trigger osmotic adjustment and other molecular events. Induction of stress-related genes also points towards a possible conservation of ABA-dependent signaling pathways in *Marchantia*. Additional molecular analyses are underway to uncover the basis of these processes at the genome-wide level.

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# Evaluation of miR-196a in the development and progression of cervical cancer

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***Introduction.*** Cervical cancer (CC) is one of the leading causes of female mortality around the world, according to the latest results of GLOBOCAN; it is the third most common gynecologic malignancy with an estimated 528,000 new cases in 2012. In Mexico, despite multiple prevention campaigns realized in order to generate awareness about the benefits offered by screening programs, such as pap smears and annual check-ups, the incidence rates have not decreased and CC remains the second most common diagnosed female cancer in the country[1]. Human Papilloma Virus (HPV) has been identified as the etiological factor in CC, however it has also been demonstrated that HPV infection is necessary, but not sufficient for the development of CC[2]. In recent years microRNAs have garnered the attention of the scientific community as a practical tool for unveiling the molecular mechanisms, in almost every known pathology. MicroRNAs are endogenous, non-coding, small RNAs which are capable of regulate the expression of genes at a post-transcriptional level by complementarity of bases with the 3'-untranslated region (3'UTR) of their targets mRNAs[3]. Deregulations of microRNAs expression profiles have been reported in many human malignancies, including CC[4–6].

***Methods.*** Using microarray technology we performed a microRNA expression profile in 35 tumor samples and 10 normal tissue samples. Our results showed that miR-196a presents an over-expression in cancer samples comparing to normal tissues ($p<0.05$). Afterwards, we employed TaqMan probes experiments with the purpose of validate these data in an independent patients cohort and also in a CC-derived cell lines panel (CaSki, C33, HeLa, ME-180 and SiHa, using HaCaT as non-tumoral control). After validation phase, we carried out the identification of the mRNA targets of miR-196a by means of the databases miRanda, miRbase and miRTarbase. We found out that Anexin 1 gene (ANXA1), Programmed Cell Death 4 gene (PDCD4), the transcription factor GATA6 and the ribonuclease III DICER1 are validated targets of miR-196a besides they are involved in the regulation of cell survival, migration and invasion in CC.

***Results.*** We found that miR-196a expression was up-regulated up to 3 times in cancer samples and up to 30 times in the CC-derived cell lines***.*** We evaluated the expression of the target genes, ANXA1, GATA6 and PDCD4 in the CC *in vitro* model and we discovered that these genes are downregulated in the tumoral cell lines (CaSki, HeLa and SiHa). We are currently testing the effect of the miR-196a inhibition in a CC cell lines panel.

***Discussion and Preliminary Conclusions.*** It has been demonstrated that aberrant expression of miR-196a affect cell proliferation, cell growth, and apoptosis in CC cell lines[3]. In our results we found that ANXA1, GATA6 and PDCD4 are downregulated in CaSki, HeLa and SiHa cell lines. As we have mentioned before, these genes are related to cellular processes such as cell migration, invasion, survival and proliferation. Together, these data lead us to hypothesize that miR-196a can modulate the development of CC through the downregulation of ANXA1, GATA6 and PDCD4.

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# Characterization of *cis*-elements that negatively regulate transcription of *EPA* genes through silencing proteins of *Candida glabrata*

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*Candida glabrata* is an opportunistic fungal pathogen capable of adhering to epithelial host cells. This adherence is mediated by some members of a large family of cell wall proteins encoded by the *EPA* (Epithelial Adhesin) genes. The majority of the *EPA* genes are localized in subtelomeric regions resulting in a negative regulation of transcription of these genes through an analogous mechanism to the subtelomeric silencing described in *S. cerevisiae*. *In vitro* adhesion to epithelial cells is mainly mediated by Epa1, the product of the *EPA1* gene which is localized at 21 kb from the right telomere of chromosome E ($E_{-R}$) and forms a cluster with *EPA2* and *EPA3*. This particular telomere contains two *cis*-acting regulatory elements: a protosilencer element called Sil2126 localized between *EPA3* and the telomere and a negative-element (NE) downstream from *EPA1*. The protosilencer Sil2126 and NE negatively regulate the expression of *EPA3* and *EPA1*, respectively. Subtelomeric silencing at this telomere depends on Rap1, Rif1 and the Sir proteins, but surprisingly not on yKu70/80 proteins.

In addition to the NE associated to *EPA1*, there are nine additional copies of the NE, some of them are associated with *EPA* genes but their function is unknown. We tested the copies of NE adjacent to *EPA6* and *EPA7* and we showed that *EPA6* and *EPA7* are negatively regulated in stationary phase by their adjacent NE. This repression is independent of the telomere proximity.

One relevant feature of the Sil2126 protosilencer is that its activity is specific to its native telomere. One possibility for this is that the NE-associated with *EPA1* located in the same subtelomeric region interacts with the protosilencer. However, we found that the NE is not required for Sil activity when we measure silencing activity of a copy of Sil2126 inserted 32kb away from the telomere.

To elucidate the silencing mechanism of the Sil2126 protosilencer in the telomere of E chromosome, a ChIP assay was used to evaluate the Rap1 protein binding to this element, since Rap1 is involved in subtelomeric silencing. Our data shows that Rap1 is bound to the protosilencer Sil2126 located between the *EPA3* and the telomere. Also, we tested a copy of Sil2126 inserted -32kb away from the telomere, a region where silencing is not present. At that distance, Rap1 is recruited by the protosilencer and is capable of mediating the silencing of the reporter gene.

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# Regulatory divergence in paralogous genes *ALT1* and *ALT2*: Rtg3-Nrg1-Gln3 negative hybrid complex regulator modulates transcriptional repression

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Genetic duplication is one of the mainly processes to generate new functions or specialized ones. After genetic duplication may occur divergence in different levels to lead the copies produced from duplication into neofunctionalization or subfunctionalization. Transcriptional diversication is one of these levels, in which duplicated genes are regulated in different manners owing to changes on the promoter or because these copies respond differently to transcription factors (TFs). *ALT1* and *ALT2* are two paralogous genes which come from Whole Genome Duplication (WGD) in *Saccharomyces cerevisiae*. *ALT1* encodes an alanine aminotransferase which translocates amino group from alanine to 2-oxoglutarate to form glutamate and pyruvate. Surprinsingly, no function has been determined for Alt2 even though these two enzymes share 67% of identity. However *ALT1* is poorly expressed in glucose-ammonium (biosynthetic conditions) and strongly induced in glucose-alanine (catabolic conditions), meanwhile *ALT2* is only expressed on biosynthetic conditions during first hours, as culture grows the expression decreases; on alanine *ALT2* expression is completely repressed. In the present work we have analyzed the transcription factors which are involved in the regulation of *ALT1* and *ALT2* expression. We found that *ALT2* is repressed for a hybrid regulatory complex integrated by three different transcription factors: Nrg1, Gln3 and Rtg3. These three TFs interact physically among them and modulate negatively *ALT2* transcription. On the other hand *ALT1* is repressed by Nrg1 only. We discovered Gcn4 is an indirect activator for *ALT2* expression and acts directly to prompt *ALT1* transcription. Also we analyzed chromatin organization on the promoters of these genes, the chromatin profile on *ALT1* promoter correlates with its expression profile; however on *ALT2* promoter the chromatin suggests a non-pivotal role to control the transcription on this gene.

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# Selection & validation of housekeeping genes and differential expression of stress related genes in *Debaryomyces hansenii* subjected to osmotic stress

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*Debaryomyces hansenii* is a marine yeast with biotechnological potential; among its attributes it has the unusual ability to grow in the presence of up to 24% w/v NaCl. Due to this characteristic, it has been classified as an halotolerant microorganism, so, *D. hansenii* is considered a suitable organism for understanding the mechanisms behind osmotolerance. There are few studies of the physiology and biochemistry of salt tolerance in *D. hansenii*, which accumulates compatible osmolytes, such as glycerol, in the interior of cells in response to high salt concentrations. A substantial amount of information of the response to hyperosmotic stress has been gathered from studies in *Saccharomyces cerevisiae*, which displays distinguishing stages as the direct consequence of the mechanical and physical forces operating. The immediate response is elicited in order to set cell protection, then to repairment and recovery, and finally to sustained adaptive events that allow restoration of cellular homeostasis under the new conditions.

Previous studies have shown that after an osmotic shock of 2 M NaCl, the expression of *D. hansenii* genes that codify for ribosomal proteins is increased significantly, and in a lesser extent, genes involved in glycerol biosynthesis. Likewise, greater expression of the CTA gene in increasing NaCl concentrations from 0 M to 0.6 M, has been observed by Northern blot analysis whereas CTT -a typical stress responsive gene- expression remains rather stable.

The aim of the present study is to gain a better understanding of the phenomena of osmoregulation and to evaluate the differential gene expression of three stress response related genes: the gene encoding superoxide dismutase (SOD), the gene for catalase A (CTA), both associated to oxidative stress, and the gene CTT linked to various types of stress (oxidative, high and low temperature, nutrient depletion, osmotic, among others). To perform the analysis by real time PCR, normalizing genes are required. Housekeeping genes (HKG) are mandatory to perform relative quantification, since their expression is stable in all experimental conditions. No reference genes have been reported for *D. hansenii* yet.

In this study, we evaluated several candidates as *D. hansenii* HKGs: phosphatidylethanolamine N-methyltransferase (FENM), an enzyme implicated in phosphatidylcholine synthesis, ribonucleotide reductase (RNR), catalyzes the formation of deoxyribonucleotides from ribonucleotides, 18S ribosomal RNA, and 40S ribosomal protein S3. Our results show that S3 is the most suitable candidate, consequently we were able to analyze the expression of three osmotic stress related genes: CTA, CTT and SOD, under four different NaCl concentrations (0, 0.6, 2, and 2.5 M). Results show that SOD and CTT transcripts have similar behavior; the absolute quantification rises in 0 and 2 M NaCl, and decreases in 0.6 and 2.5 M NaCl. CTA expression increases proportionally with the salt concentration.

# Biochemical characterization of the putative protein Rad51 from *Milnesium tardigradum*

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Tardigrades are cosmopolitan invertebrates, and as exceptional animals they have adapted to the most extreme environments surviving by using two types of dormancy: quiescence with various forms of cryptobiosis and diapause during any stage of its life cycle, from egg to adult. Individuals which are frozen or desiccated show a very high tolerance to a number of other non-natural conditions, including exposure to ionizing radiation and immersion in organic solvents.

*Milnesium tardigradum*, one of the most resistant species, belongs to the class Eutardigrade, order Apochela and family Milnesiidae. This tardigrade is able to survive to high doses of ionizing radiation in hydrated and anhydrobiotic state. However as shown by a comet assay the integrity of the *M. tardigradum* genome is compromised if the anhydrobiosis state is prolonged and it has been shown that the damage inflicted on the DNA is time dependent. Consequently *M. tardigradum* requires the participation of efficient mechanisms for DNA repair after leaving the anhydrobiotic process. One of the most efficient mechanism is the homologous recombination in which Rad51 is the main recombinase. This protein is able to bind single (ssDNA) and double stranded DNA (dsDNA), and carries out ATPase function.

Recently, rad51 gene from *M. cf. tardigradum* was identified and its sequence contains conserved domains: Walker A and B, L1 and L2 and ATPcap characteristic of the recombinases family. Consistently, the predicted structure of MtRad51 overlapped that of Saccharomyces cerevisiae crystallized Rad51 protein. Further, the use of heterologus antibodies against Rad51 in *M. tardigradum* exposed to gamma radiation, revealed that Rad51 (MtRad51) proteins levels were highly induced. However, it is necessary to evaluate the MtRad51 catalytic properties.

To achieve this, in this work, we cloned, expressed and purified MtRad51. For this, rad51 cDNA from *M. cf. tardigradum*, was cloned in the plasmid pProex-1 that bears a 6Xhistidine tag which allowed its purification by Nickel resin after the induction of its expression by IPTG. Then, purified recombinant rMtRad51 protein was used to evaluate its ability to bind labeled ssDNA and dsDNA probes by Electrophoretic Mobility shift assay (EMSA). Our results indicate that the purified rMtRad51 binds dsDNA and ssDNA. ATPase activity is currently being evaluated. These experimental evidence will allows to confirm that MtRad51 is a *bona fide* recombinase.

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# Genome-wide profiling of DNA methylation in response to resveratrol identified novel therapeutic epigenetic targets in breast cancer cells

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**Introduction:** Aberrant DNA methylation is a frequent epigenetic alteration in cancer cells that has emerged as a pivotal mechanism for tumorigenesis. A great interest has been focused on natural compounds as epigenetic modulators or “epidrugs” in tumor cells that are being explored with the aim to restore normal DNA methylation on oncogenes and tumor suppressor genes. A limited number of studies indicate that the dietary compound resveratrol modulates DNA methylation of several cancer-related genes; however a complete view of methylation changes in epigenome after resveratrol treatment have not been reported yet in cancer.

**Results:** In this study we performed a genome-wide survey of DNA methylation in triple-negative MDA-MB-231 breast cancer cells exposed to resveratrol using thearray-based profiling of reference-independent methylation status followed by whole-genome hybridization of human DNA methylation promoter microarrays. Interestingly, resveratrol treatment for 24 h and 48 h induced a gradual decrease in promoter DNA hypermethylation and an increase in DNA hypomethylation in comparison to control cells. Our data indicate that in control non-treated cells 2,476 and 1,017 gene promoter loci were hypermethylated and hypomethylated, respectively. Of total 2476 hypermethylated genes, 1,459 and 1,547 were differentially hypomethylated after 24 h and 48 h treatment, respectively, in comparison to non-treated cells. Remarkably, resveratrol did not induced widespread non-specific hyper or hypomethylation as changes in methylation were found in only 12.5% of 27,728 CpG loci studied. Moreover, resveratrol was able to restore the hypomethylated and hypermethylated status of key tumor suppressor and oncogenes, respectively. Integrative genomic analysis of methylome and transcriptome profiles indicates that DNA methylation alterations were concordant with changes in mRNA expression of a group of oncogenes (AURKA, CCNB1, HK2, MMP9, RUNX2) and tumor suppressor genes (SLIT3, SLC27A2, AMY2A).

**Conclusions:** Our findings not only reveal for the first time the genome-wide impact of resveratrol on methylome of breast cancer cells, but they also identify novel potential targets for epigenetic therapy. Although speculative, we propose that resveratrol modulate gene expression and exerts anti-tumor activities based on its ability to modify the DNA methylation status of cancer-related genes.

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# Establishment of molecular basis of the classification of histone deacetylase

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Nucleosome consists of an octameric core composed of two copies each of the four histone proteins: H2A, H2B, H3 and H4. The histone proteins are chemically and reversely modified mainly in the amino-terminal region. These modifications include phosphorylation, ubiquitinylation, methylation, sumolyation, acetylation, among others. Histone acetylation occurs at lysine residues by the action of the histone acetyltransferases, while histone deacetylases (HDACs) remove the acetyl groups from histones and this effect is related to repressive transcription.

HDACs from model organism have been previously classified according to the homology with HDACs from yeast and human. Therefore there is no clear molecular basis for the classification of HDACs in eukaryotes. This study aims to establish a molecular classification based on the domains and motifs of amino acid sequences of eukaryotic HDACs.

In this work, we analyzed 529 HDACs, obtained by HMM profile. The sequences were analyzed and classified into two groups based on their different catalytic mechanism. HDACs dependent on $Zn^{2+}$, which include classes I, II and IV. HDACs that require Nicotinamide adenine dinucleotide ($NAD^{+}$) as cofactor, named class III or sirtuins. In addition, plants contain a unique type of HDACs called histone deacetylase 2 (HD2).

This study will aid in allowing us to define the differences in the catalytic mechanisms and subcellular localizations of HDACs and also to detect functional regions of each group of HDACs established.

In addition, the enzymes with hypothetical or putative description could be grouped with perfectly annotated as protein sequences and experimentally demonstrated catalytic function, which allowed the characterization of unannotated proteins.

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# Expression pattern of miRNAs associated with the response to conventional treatment in patients with cervical cancer

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**Introduction**. Micro-RNAs or miRNAs (approximately 20-25 nucleotides long) play an important role during multi-step carcinogenesis. miRNAs have been implicated in the regulation of several biological process. Interestingly, several miRNAs have been implicated in the control of genes that participate in apoptosis (miR-15 y miR-16), cell-cycle (miR-221, let-7a, miR-21, miR-34a), and metastasis (miR-10b y miR-183). Recently, they have been associated to radio-resistant phenotype in lung and breast cancer cell lines (miR-126, miR-let-7a, miR-49). The aim of this work was to find miRNAs that could discriminate radio and chemo-resistant phenotype by means of miRNA profiling in Locally Advanced Cervical Cancer patients (CCLA).
**Methods.** We analyzed the human miRNome (miRBase V.16) by qPCR plates in tumor samples biopsies (CCLA), treated by conventional therapy (Radiation based concomitant with cisplatin). Samples derived from patients were divided into two groups according to clinical outcome (complete response vs. No response to conventional treatment).
**Results.** We found 101 miRNAs that were significant differentially expressed ($p< 0.05$) between both samples groups (4 over-expressed and 97 down-regulated). Moreover, miR-144, miR-3176, miR-31-3p y miR-3676 were found over-expressed in radioresistant tumor samples and markedly miR-1, miR-10b, miR-10a, miR-100 y miR-204 were down-regulated (up to 15 times in comparison to complete response samples). The identified miRNAs in this study have been associated with three novel molecular pathways (Jak-STAT, Notch y ErbB) with no previously association with the acquisition to radio and chemotherapy in CLLA patients. We considered that early detection of these miRNAs could be used as a predictor outcome in cancer cervical patients.

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# DNA methylation profiling at a single locus of GNPDA2, PPARGC1α and LEPR genes from umbilical cord and the correlation analysis to maternal-fetal anthropometry in pregnancy.

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# An adenosine derivative compound exerts a hepatoprotective effect involving epigenetic changes in $CCl_4$ – induced rat cirrhosis

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Pathological characteristic of cirrhosis is scarring generation that results in a structural distortion and dysfunctional liver. Transcriptome analysis performed in our laboratory showed that differential genes deregulated in $CCl_4$-induced cirrhosis tended to normalize their expression by treatment with IFC-305, an adenosine derivative. Among them two genes involved in fibrogenic process, *Col1a1* and *Pparg*. With the aim to understand such differences we asked whether epigenetic events are responsible of those changes in fibrosisrevertion by IFC-305 compound.

Wistar rats were treated with $CCl_4$ and a group of themwas administered with IFC-305 to reverse fibrosis phenotype. In both conditions, DNA methylation and histone modifications were evaluated globally and, locally at the promoter regions of*Col1a1* and *Pparg*genes.

Global DNA methylation analysis in cirrhotic rats showed thatIFC-305 triggers a DNA methylationand reestablised normal levels of 5hmC and H4ac. Afterwards, we analysed locally histone post-translational modifications and found that in a cirrhotic state there is a compact-chromatin context at *Pparg*gene promoter, while administration of IFC-305 brings to an open-chromatin related with overexpression of gene. Concerningto DNA methylation status, we found that 4 dinucleotidesCpG in promoter region of *Col1a1*, loses methylation on cirrhosis and correlating with increased synthesis of collagen I. In contrast, IFC-305 increased DNA methylation around transcription start site, and this can be related with diminishment of collagen I along cirrhosis reversion.

Here we proposed that IFC-305-fibrosis reversion treatment involves global chromatin changes and epigenetic regulation on*Col1a1*and *Pparg*gene promoters.

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# Profile of cytokine/chemokines associated to Th17 response as distal progression markers in patients with Locally Advanced Cervical Cancer (LACC).

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**Abstract.** The cervical cancer (CC) is one of the public health problems worldwide of first order that must be addressed comprehensively and inter form. In Mexico, the CC it is the third most prevalent cancer and the second most common in women. Given the dificiencies in the timely diagnostic systems, more than 80% of patients are diagnosed in advanced stages, and are classified as Locally Advanced Cervical Cancer (LACC). The prognosis for patients who develop recurrence is extremely poor, with a survival rate at one year between 15 and 20%, considered one of the leading causes of death from this disease. At present, they have developed research strategies focused on the research and identification of biomarkers for early diagnosis of susceptibility and development of recurrence, progression and metastases. Soluble immunological mediators associated to specific cellular responses are cytokines and chemokines; many of these molecules have been reported in different stages of CC development. The predominant type of immune response in cervical cancer depends on the type of cytokines expressed and the cross-talk between tumor-microenviroment. OBJETIVE. In the current research, the main important point was aimed to evaluate the level of participation of the cytokine, chemokines and their receptors associated to specific immunological response and establish and expression profile in a cohort of 89 patients diagnosed as LACC. MATERIAL AND METHODS. First, we used a data base of genome-wide high-density microarrays reported by our group. Differentially expressed genes in tumor tissues (TT) were validated and avaluated by quantitative RT-PCR (10 genes) and Immunohytochemistry (5 genes) in a new cohort of 20 TTs of patients diagnosed with LACC, as controls we evaluated the expression levels in 10 Normal Tissues (NT). RESULTS. Statistial analysis shown that 84 genes are differentialy expressed in tumor tissues, 23 over-expressed and 61 down-expressed (ANOVA, $P<0.05$). Interestingly, 7 and 6 over-expressed genes of cytokines/receptors and chemokine/receptors have been reported as inducing and effector molecules associated to Th17 immunological response respectively. By qPCR validation, 6 ligands of cytokine (3 inducing molecules, IL-6, IL-18 and IL-17F) and chemokines (3 effector molecules, CXCL1, CXCL10 and CCL20) were present in a new cohort of 20 TT of patients. In the case of inmunohistochemical staining we validated the presense of 5 receptors (IFNγRa, IL4-Ra, TGFβRII, IL-17RC, CCR7).These molecules can act as inducers of the response, as well as effector of Th17 immunological response. CONCLUSIONS. Over-expression of ligand and receptors of cytokine and chemokines establish a suitable tumor microenvironment to induces expression and predominance of molecules associated with Th17 immune response in patients diagnosed as Locally Advanced Cervical Cancer (LACC), The presence of this microenvironment, may be associated with the susceptibility of some patients to present recurrence and progression, which are manifested by metastatic disease, this proposal is currently under study.

# Cytotoxic Effects of Avocado Lipids (*Persea americana* var. drymifolia) on Cancer Cells

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Cancer is a leading cause of death worldwide. In 2012, cancer caused 8.2 million deaths, and cancers of the lungs, liver, colorectal, stomach, and breast are main types. In Mexico (2012) about 70,000 deaths were reported for this disease, being breast, prostate, cervical and colorectal cancer the highest incidence. A hallmark of cancer is the rapid growth of abnormal cells that extend beyond their usual limits and invade adjoining parts of the body or spread to other organs, a process known as metastasis. Cancer treatment requires careful selection of one or more therapeutic modalities, such as surgery, radiotherapy, or chemotherapy. Despite progress in anticancer therapies, the chemotherapeutic drugs used in cancer treatment have the serious drawback of nonspecific toxicity. Additionally, many neoplasms eventually become resistant to conventional chemotherapy because of selection for multidrug-resistant variants. These limitations have led to the search for new anticancer therapies. It is known that some fatty acids are cytotoxic against cancer cells. However, little is known about the cytotoxicity of lipids [FA and fatty acid derivatives (FAD)] from the Mexican criollo avocado seeds (*P. americana* var. drymifolia). In this study we assessed the cytotoxic effects of FA and FAD from avocado on three cancer cell lines (HeLa, MCF-7 and Caco-2), and a primary culture of bovine mammary epithelial cells (bMEC). Avocado lipids were obtained from seeds with hexane and characterized by CG-MS. By MTT and trypan blue exclusion assays we showed that avocado FA and FAD (75, 100 and 150 μg/ml) were cytotoxic to all of the cells evaluated in a concentration-dependent manner. Also, avocado FA and DAG inhibited the cellular proliferation (20 μg/ml). Additionally, by flow cytometry we showed that avocado FA and FAD (75 μg/ml) induced apoptosis in Caco-2 cells and bMEC. In conclusion, avocado FA and FAD are cytotoxic to all cell types tested, which was concentration-dependent.

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# "Characterization of the antimicrobial activity of a recombinant 110-kDa membrane protein produced by *Pediococcus acidilactici* ATCC 8042".

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*Pediococcus acidilactici* ATCC 8042 is a non-pathogenic strain that has been used as a starter culture in fermented meat products, where its biopreservative effect has been observed as well as the growth inhibition of *S. aureus* and mesophilic bacteria (Rivera , 2004 ). This phenomenon is not attributed to pediocin production because the strain lacks the genes for the production of the main bacteriocins synthesized by this genus (Mora *et al*., 2000; Mora *et al.,* 2003).

The production by *P. acidilactici* ATCC 8042 of two membrane proteins of 99 and 110-kDa which exerted antibacterial activity, shown in agar diffusion tests and zymograms, was previously reported by this group (García Cano *et al*., 2011). Both proteins were copurified and identified. The results indicated that the 110-kDa protein presents the characteristic regions of a membrane protein with unknown function, with a 47 % coverage and 100 % identity. Therefore, the antimicrobial effect exerted by this protein represent an enigma, because its sequence is not similar to known antibacterials. This work is aimed at solving some of the main questions about the mechanism of inhibition thereof.

To analyze the individual antimicrobial activity of the 110-kDa protein of *P. acidilactic*i *ATCC 8042*, the gene for its expression was cloned in a heterologous system consisting in pET19 as a vector and *E. coli* BL 21 as a host. The recombinant protein showed the expected molecular weight and produced observable antimicrobial activity on agar diffusion tests and zymography. A bioinformatic analysis of the amino acid sequence of this protein was also performed to determine the secondary structure and topology of the protein in the membrane, which is formed by two regions: a highly conserved transmembrane domain and a poorly conserved region located outwards of the cell and in contact with the peptidoglycan layer. New sequence analysis indicates its similarity to a phage infection protein type YhgE, which has been reported as a structural gene in the pili operon in *Lactococcus lactis* (Oxaran et al., 2012). Homology modeling allowed to visualize that the three-dimensional structure of the protein showed similarity with a colicin type IA (bacteriocin of *E. coli* ) whose mechanism of action involves the formation of pores in the membrane.

# Effect of Immunomodulatory Molecules on the Gene Expression of *Staphylococcus aureus* Virulence Factors: Implications During Bacterial Internalization Into Bovine Mammary Epithelial Cells

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*Staphylococcus aureus* is an intracellular facultative pathogen of human and animals responsible of chronic diseases (i.e. subclinical bovine mastitis). This pathogen have a wide diversity of virulence factors (i.e. *agrA, RNAIII, clfB, fnbA*), some of which favor its internalization into nonprofessional phagocytic cells, like bovine mammary epithelial cells (bMECs). In a previous study, we demonstrated that some immunomodulatory molecules, such as fatty acids and vitamins, reduce the internalization of *S. aureus* into bMECs improving the host innate immune response. However, the effect of these compounds on the gene expression of *S. aureus* virulence factors is unknown, which was the aim of this study. For this, we used a *S. aureus* strain (ATCC 27543) that is able to internalize into bMECs. To evaluate the mRNA levels of the virulence factors (*agrA*, *clfB, RNAIII* and *fnbA*), *S. aureus* was cultured in LB medium and sodium butyrate (NaB, 0.5 and 2 mM), sodium octanoate (NaO, 0.25 and 1 mM) or vitamin $D_3$ (vitD, 50 and 200 nM) were added for 2 or 24 h. Next, RT-qPCR was performed and *16S* gene was used as endogenous gene. Also, we evaluated the *S. aureus* internalization into bMECs by gentamicin protection assays. The results showed that both concentrations of NaB (2 h) down-regulated the mRNA level of *clfB* (~0.5-fold); meanwhile, the *clfB* gene expression was increased (~2.8-fold) at 24 h, which correlated with decreased internalization (40%). On the other hand, NaO (0.25 mM, 2 h) slightly up-regulated the mRNA level of *clfB* and inhibited the bacterial internalization (30%). We did not observe a correlation among the internalization of *S. aureus* and the up-regulation of *clfB* and *agrA* expression modulated by 0.25 mM NaO (24 h). The 50 nM vitD treatment (2 and 24 h) increased the mRNA level of *RNAIII* (~5 and ~2-fold, respectively). In addition, 200 nM vitD (24 h) up-regulated the *RNAIII* and *clfB* gene expression, which correlated with the inhibition of bacterial internalization (40%). In conclusion, the short chain fatty acids and vitamin $D_3$ are able to modulate the gene expression of *S. aureus* virulence factors. Additionally, the modulation of the (i) *clfB* gene expression by fatty acids or (ii) the *RNAIII* and *clfB* gene expression by vitD correlated with the inhibition of internalization of *S. aureus* into bMECs.

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# Cockatiel ( *Nymphicus hollandicus* ) gut microbiomes, bacterial inhabitants of a worldwide distributed pet

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Avian gut microbiomes models are relatively few and they focused in poultry looking improve their health and weight gain, but there is little information about microbiome in pet birds. Psittacids (parrots), are very common as pets around the world, among these birds one of the most sold is the specie *Nymphicus hollandicus* (cockatiel). The compositions of the microbiome of these birds are important due to the close relations between humans and pet birds, it also shows the composition of both cultivable and uncultivable bacteria, allowing us a new perspective between what is considered healthy and sick. Understand the composition of the intestinal microbiome of the pet birds result fundamental, because it tells us that bacterial groups are essential for the maintenance of metabolic state.

To understand the composition of intestinal microbiome three healthy *N. hollandicus* living in captivity were sampled by cloacal swabs and the bacterial composition was determined by massive 16S ribosomal gene sequencing. The assignment of Operational Taxonomic Units (OTUs) was made by BLAST against Greengenes data base, all statistical and diversity analysis were performed by platform R. Microbiome data were compared with other grain-eating birds microbiomes to understand the development of the gut microbiome of the cockatiel.

A total of 295,217 sequences were grouped into 18,280 bacterial OTUs. The family *Erysipelotrichaceae* represents the 57 % of the all sequences. Comparing with other birds like turkeys and chickens, the microbiome of the cockatiel is similar in the dominance of *Firmicutes* and similar abundances with those of Proteobacterias. We search for pathogenic organisms for birds and people; we found that some pathogen species form part of the microbiota naturally of *N. hollandicus* while other pathogens were found in low numbers. This work is the first for *N. hollandicus* gut microbiome.

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# Phenotypic plasticity of *Bacillus* isolates from Coahuila, Mexico.

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One common phenomenon in organismal biology is phenotypic plasticity: the capacity of a given genotype (or individual) to change its phenotype in response to a change in the environment. Phenotypic plasticity determines tolerance ranges of species when facing environmental challenges. Reaction norms can be applied in evolutionary biology to characterize phenotypic plasticity in a diversity of traits. The component functions of reaction norms can themselves be considered as traits of an individual or genotype. Understanding the causes and consequences of individual or species differences in phenotypic traits is of high importance to evolutionary ecology.

Strains of the genus *Bacillus* were isolated from the Churince, Cuatro Ciénegas, Coahuila, Mexico. These have shown to have different qualitative phenotypes between species at a morphological and physiological level but quantitative phenotypes, as norms of reaction, might also be a species trait and be explained by evolutionary history. Since the different *Bacillus* species isolated from the Churince pond have shared the same physical-chemical conditions for hundreds of years, we have a chance to ask whether different isolates/species exhibit differences in their phenotypic plasticity to these environmental parameters. In this work, we determined the norms of reaction for six strains of *Bacillus subtilis* and six strains of *Bacillus cereus* measuring growth rate and survival to different environmental factors (temperature, concentration of NaCl and UV radiation). Regarding UV-light resistance, thus far, we did not found differences between the two species, as all the strains tested showed similar survival rates. Temperature appears as one of the factors of greatest effect on the ecology and adaptation of organisms, including bacteria. We found that the *subtilis*-species tolerate higher temperatures (growing at 47°C and even 52°C) as compared to the *cereus*-species for which growth rate significantly decreases at temperatures above 47°C. The results showed that for temperature, reaction norms grouped the *Bacillus* species in a similar manner as taxonomic techniques (morphology, physiology, and biochemical tests), the phenotypic plasticities showed strong evidence of differential adaptive characteristics associated with evolutionary history. We will discuss this and other data regarding reactions norms for the natural *Bacillus* spp. isolates.

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# Proprotein Convertase Selectivity in the Activation of the Human Papilloma Virus

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The family of host cell serine proteinases, known as proprotein convertases (PCs), is exploited by a variety of human pathogenic viruses to promote viral activation. The PCs, furin, PC5, PACE4, and PC7 reside in the trans-Golgi network membranes of the constitutive secretion pathway, and are differentially expressed in most human tissues. We study PC selectivity for the activation of the human papilloma virus (HPV), and for that purpose we applied an approach that combines the use of recombinant PCs, an assay of HPV cell entry, and the kinetics of inhibition of PCs by serpins. We found differences in PC selectivity between mucotropic HPV genotypes 16 and 18 for activation by furin, PACE4, and PC7, and showed that both viral genotypes are inactivated by PC5. These findings imply that proteolytic cleavage of the coat proteins in these two high cancer-risk HPV genotypes involves multiple cleavage sites with different PC specificity, and challenge the current accepted model of HPV cell entry that postulates that only one PC cleavage site, located on the coat protein L2 at position Arg12, is needed for viral activation. The inactivation by mutagenesis of the six potential PC cleavage site motifs in the coat proteins of HPV16, two in L1 and four in L2, demonstrated that additional PC cleavage sites in both coat proteins are required for HPV16 infection. Results are discussed from the perspective that PC selectivity could be a determinant of viral tropism to anatomical sites of infection based on differences in tissue PC expression profile.

# Influence of resveratrol in the chronological life span of *Saccharomyces cerevisiae*

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A broad range of health benefits has been attributed to the diet supplemented with resveratrol (3, 4, 5-trihydroxystilbene, RSV) in mammalian models, including the extension of longevity. Nonetheless, despite the growing number of RSV studies in the biomedical area, the mechanism by which the RSV acts remains unknown. Recently, it has been proposed that the inhibition of the oxidative phosphorylation is the molecular target of RSV. This mechanism suggests that the RSV may cause mitochondrial dysfunction and oxidant damage to cells with a concomitant decrease of the cell viability and cellular life span. To prove this hypothesis we decided to use the chronological life span (CLS) of *Saccharomyces cerevisiae* that has been accepted as an important model of oxidative damage and ageing; as well as oxygen consumption, mitochondrial membrane potential and hydrogen peroxide ($H_2O_2$) release to study mitochondrial dysfunction. We found that the supplementation of *S. cerevisiae* cultures with 100 µM RSV decreased CLS in a glucose-dependent manner. Indeed, the RSV supplementation also affected oxygen consumption in a glucose-dependent shape; at high glucose level (10% *w/v*), RSV prompted oxygen consumption and in the low glucose level (0.5% *w/v*), RSV inhibited the oxygen consumption. Unexpectedly, we found that supplementation with 30 and 50 µM RSV in cultures grown with the high-level of glucose, increased the mitochondrial membrane potential; whereas, in the low-level of glucose, only the supplementation with 100 µM RSV increased the mitochondrial membrane potential. Finally, the RSV supplementation decreased the $H_2O_2$ release at high glucose and increased it at low glucose level. Altogether, this data support the hypothesis that RSV supplementation decreases the CLS of *S. cerevisiae* and this might be caused by mitochondrial dysfunction.

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# The microbiome of CAM plants: diversity and functional strategies in drylands

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## Abstract

Cacti and Agaves represent keystone species in arid and semi-arid ecosystems. Both plant families originated in the American continent and share the Crassulacean Acid Metabolism (CAM) for fotosynthesis. Despite their ecological relevance, little was known about the microbial communities associated with them, the factors that most influence their assembly, their diversity, and ultimately their function and contribution to plant fitness and adaptation.

By phylogenetic profiling, we identified the prokaryotic and fungal communtities associated with cultivated *Agave tequilana* and wild *Agave salmiana, Agave deserti, Myrtillocactus geometrizans and Opuntia robusta* in the rhizosphere, phyllosphere, root and leaf endosphere, and bulk and proximal soil across their natural habitats expanding from California to Central Mexico[1,2,3]. Our analyses revealed that prokaryotic communities were mostly influenced by the plant compartment, whereas biogeography played a larger role in the fungal assemblies. Interestingly, prokaryotic diversity was similar in both the rhizosphere and phyllosphere of wild CAM species, but strongly reduced in the cultivated *A. tequilana*[2].

Moreover, our dataset allowed us the identification of key microbial taxa that are common to the five CAM plants analyzed to date, and by using culture-based methods, we isolated, identified and characterized some of them. We have shown that several of these bacterial and fungal strains possess traits that promote plant growth and tolerance to drought[1,2,3]. Currently, we are evaluating their functions *in planta* using a variety of approaches.

Altogether our work supports the notion that desert CAM plants establish similar plant-microbe interactions, which are important for their success in drylands.

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# Dynamics of the actin cytoskeleton and vesicle traffic in the lack of MYO-5 in *Neurospora crassa*

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In filamentous fungi, polarized growth is the result of vesicles secretion at the hyphal apex. Vesicle transport to its target destination in plasma membrane is mediated by motor proteins via actin and/or microtubular cytoskeleton. Motor proteins associated to actin filaments are known as myosins. Specifically, class V myosins are responsible for the intracellular transport of cargo in different organisms. Previously, we studied the dynamics and localization of the only class V myosin in *Neurospora crassa* tagged with GFP. In this study, we are showing the effect of the lack of MYO-5 in the organization of the actin cytoskeleton and vesicles containing chitin synthases. In a deletion mutant of the *myo-5* gene, we tagged actin with the reporter Lifeact-GFP and the CHS-1 (Chitin synthase-1) and performed confocal and TIRF microscopy. In the Δ*myo-5* mutant, we observed that actin cytoskeleton is not well organized in the apex and the subapex. We observed thick actin cables in the cytoplasm and a concentration of actin in the contour of the apical dome. Nevertheless, we did not observe the subapical endocytic collar or the accumulation in the Spitzenkörper (Spk). In conidia, the actin filaments form a ring that last until germination. Vesicles containing CHS-1-GFP, had a different pattern in the Δ*myo-5* mutant. There was no concentration of CHS-1-GFP in the Spk, instead there is a cloud of fluorescence occupying the apical dome. Although, CHS-1-GFP was well organized in septa as in the wild type strain. These results suggest that MYO-5 plays an important role in the apical organization that supports morphogenesis and growth in the filamentous fungus *N. crassa*.

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# Characterization of human papillomavirus type 33 variants circulating in San Luis Potosi, Mexico

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Cervical cancer (CC) is the second cause of death by cancer among Mexican women. In Mexico, 4,031 deaths due to CC were reported in 2008 and the mortality rate was 9.7 per 100,000 women, around three times higher than in developed countries. Persistent infection by human papillomavirus (HPV) induces neoplastic lesions that may progress to invasive cancer. Among the 40 anogenital HPV types, low-risk (LR-HPV) types are associated with benign tumors and high-risk (HR-HPV) types with CC. The HR-HPV types HPV16 and HPV18 cause around 70% of CC cases worldwide, whereas the LR-HPV types HPV6 and HPV11 cause 95% of the anogenital wart cases. A previous study of our group identified 13 HR-HPV and 6 LR-HPV types in cervical scrapes of women sampled from 2007 to 2012 in 49 municipalities of the state of San Luis Potosí. HPV infections were found in two-thirds of them, with HR-HPV types being three times more prevalent than LR-HPV types. The predominant type was HPV33 whose prevalence (33%) doubled that of HPV16, the second most prevalent type. Association of all HR-HPV types increased as a function of the severity of neoplastic lesions except for HPV33, whose prevalence was higher in low-grade squamous intraepithelial lesions (LSIL). These findings suggested that an HPV33 outbreak was taking place at the time of sampling. In order to identify the circulating HPV variants and to determine their geographic distribution, we determined the prevalence of HPV33 single infections among municipalities and sequenced the LCR-E6 genomic region in 114 of them. HPV33 single infections predominated in the Middle, Center and Huasteca zones, whereas HPV16 single infections predominated in the Altiplano zone; the highest HPV33 prevalence occurred in municipalities of the Middle zone. Most of the high-grade squamous intraepithelial lesions (HSIL) associated to HPV33 single infections were located in the Huasteca zone. Different oligonucleotide combinations were used to obtain amplicons with overlapping sequences covering the HPV33 LCR-E6 region. In some cases where no amplicons were obtained, preamplification by rollling circle amplification of cervical samples to increase HPV33 episomal forms led to obtaining complete LCR-E6 amplicons with a single oligonucleotide pair. All 82 LCR-E6 complete sequences obtained correspond to the HPV33 A1 sublineage; 80 sequences were identical and two had single nucleotide polymorphisms in nucleotides 485 and 582 of the viral genome resulting in amino acid changes of the E6 oncoprotein.

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# Novel microbial species isolated from subaerial biofilms of stone monuments

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Subaerial biofilms are complex microbial communities composed by heterotrophic and phototrophic microorganisms. These communities develop on mineral surfaces, such as rocks, continuously exposed to the atmosphere. Since most of the Tangible Cultural Heritage is made of rock, the stone monuments and some engineering-works are niches on which the subaerial biofilms commonly develop damaging the structure. Mexico possess a large number of archaeological zones and monuments, catalogued as World Heritage, along the national territory. Some of those monuments have been exposed to the atmosphere during centuries developing centennial biofilms and others, although they were built centuries ago, have been re-exposed recently developing younger biofilms; therefore the subaerial biofilms characteristics are variable among those monuments. There are just a few studies about the microflora that develops on those structures, so these niches are a source of novel and poor characterized microorganisms with biotechnological potential. In this work, it is described three novel microorganisms isolated from a subaerial biofilm from a stone pyramid in the archaeological zone of "*Cañada de la Virgen"*: one cyanobacteria, one microalgae and one fungus. A polyphasic characterization was done using molecular, morphological and physiological data. Phylogenies were constructed using 16S, 18S and ITS sequences, according the respective taxa. Micro and macro-morphological characteristics were determined using light microscopy and SEM. Physiological traits as secondary metabolites production were tested using GC-EIMS. In conclusion, we found one novel cyanobacterial species of the *Nodosilinea* genus, one novel green-algal species of the *Chlorosarcinopsis* genus and one novel fungal species not belonging to any previously reported genus.

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# Killer peptide targeting arrested cells.

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The capacity to induce cell death in cells under cell-cycle arrest has implications in understanding both basic aspect of cell physiology and in the development of a novel therapy against resilient cancer and infections resistant to common antibiotics. Iztli peptide 1 (IP-1) has two activities, the α-pheromone and antimicrobial activities. IP-1 induces cell death specifically on *Saccharomyces cerevisiae* mating type A cells (MatA), and this cytotoxicity depends on the α-pheromone-signaling pathway. The pheromone pathway involves the mitogen activated protein kinases (MAPKs) pathway, a conserved pathway that controls cell cycle progression in mammalian and yeast cells. This suggests that IP-1 may be a good model to study the relationship between cell death and cell cycle arrest both in microbes and mammalian cells.

We observed that knocking out the gene leading to cell cycle arrest in the pheromone pathway (*FAR1*) in MatA cells made them resistant to the IP-1 toxicity. Matα are naturally resistant to the cell death induced by IP-1 because these lack the α-pheromone receptor (*STE2*), yet knocking out the mitogen-activated protein kinase *HOG1* in these cells induces cell-cycle arrest in high osmolarity and IP-1 then kills these cells as well. Finally, using an inhibitor of the cyclin-dependent kinase (CDK) catalytic subunit, *CDC28,* that have been shown to induce cell-cycle arrest independent of any signaling also makes cells susceptible to the killing of a variant of IP-1 which does not activate the pheromone pathway.

In summary, our results indicate that IP-1 induced cell death on yeast by a mechanism that depends on the cell-cycle arrest.

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# Autophagy and senescence during the development of spinal cord and the differentiation of motoneurons

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During embryonic development about 50% of the initially generated motoneurons die by programmed cell death. Most of the cells are eliminated though apoptosis, but other mechanisms exist to control the final cellular number. We propose here that one of these mechanisms is autophagy, a lysosomal degradation pathway. Autophagy may influence cell fate by promoting type II cell death or modulating senescence. Autophagy inhibition in explants of developing spinal cord resulted in an increased number of motoneurons without an equivalent reduction of apoptotic cell death. This suggests that autophagy regulates the number of motoneurons by inducing type II cell death. In addition, another way that autophagy might influence cell number is by modulating developmental senescence of a subpopulation of motoneurons, which may be cleared by phagocytosis. In fact, regions of the mouse embryo that show developmental senescence overlap with regions containing cells undergoing apoptosis and cells with abundant acidic vesicles, suggesting autophagic activity. We will show that both autophagy and senescence are indeed observed in the region corresponding to motoneurons. In contrast, the floor plate appears rich in senescent cells but lacks autophagic activity. We discuss whether autophagy and senescence occur independently during the differentiation of motoneurons.

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# Defective Autophagy in Neuronal and Glial Senescence

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Cellular senescence is a biological state induced by various stressful stimuli including telomere dysfunction, reactive oxygen species, DNA damage, oncogene activation and developmental clues. Senescence phenotype is characterized by an irreversible cell cycle arrest, senescence-associated β-galactosidase activity (SA-β-gal), senescence-associated heterochromatin foci, DNA damage foci and accumulation of lipofuscin. In ageing, senescent cells are not efficiently cleared by the immune system leading to an accumulation of these cells in various aged tissues, where they express a complex senescence-associated secretory phenotype (SASP), which is harmful in long term. The SASP includes inflammatory cytokines, chemokines, growth factors and proteases and is proposed to underline age-related diseases. Cellular senescence has been described in mitotic cells, and it has been assumed that post-mitotic cells are incapable of entering into a senescent state. Nevertheless, neurons with several senescent features have been observed in old mouse brain. To study the molecular mechanisms of both neuronal and glial senescence establishment, we developed an *in vitro* model of senescence of primary cultures of rat cortex. We found that during neuronal and glial senescence autophagy flux is impaired. Autophagy is a catabolic process that, through lysosomes, degrades intracellular components into basic biomolecules. Our findings suggest that defective autophagy would contribute to neuronal and glial senescence.
**Key words:** cortex; neurons; glia; autophagy; aging; cellular senescence; SASP; mammals
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# Molecular analysis of the process of sarcopenia: Changes in muscle gene expression between young and older adults

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**Background:** One of the deleterious effects of aging is loss of muscle mass; phenomenon known as sarcopenia. This condition is highly prevalent in older adults being the major cause of disability. In the coming decades, the change in the population pyramid will result in a high prevalence of disability and decreased quality of life because of sarcopenia. The etiology of sarcopenic process has not been clearly established, however is considered as a progressive and multifactorial impairment. It can start from the fourth decade of life, directly affecting the volume, muscle strength, and function until the fragile state of the elderly. **Objective:** With the aim of studying and understanding the molecular mechanisms involved in the sarcopenic process, we analyzed expression changes between older adults and young people, of the signaling pathways involved in muscle maintenance. **Methodology:** gene expression patterns of fourty muscle biopsies (femolar quadriceps) in four groups; functional elderly, frail elderly, sedentary young and athletes young, were analyzed using QRT-PCR low density arrays (RT2 Profiler, Qiagen). The signaling pathways involved in the process sarcopenic (apoptosis, autophagy, WNT, TGF-$\beta$, TNF-$\alpha$ and interleukins) were studied. We used immunohistochemistry to assessed the differences in the distribution and expression of muscle proteins involved in the contractile process (actin and myosin). **Results:** The genetic profiles showed an increase in genes involved in apoptosis and inflammation in the group of frail elderly. Surprisingly, gene expression of functional elderly was very similar to young sedentary, while athletes showed expression of genes related to autophagy. Immunohistochemical analysis showed a change in the distribution of actin and myosin in fragile elderly. Our data suggest that in the frail elderly, the inflammatory and apoptotic process in muscle fiber have a direct impact on the proteins involved in contractility and muscular functionality, which it is clinically reflects as lower volume, muscle strength and performance in this population

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# Identification of potential pathogenic microorganisms in the tract of nymphs and canaries in captivity.

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In Mexico, the commercial breeding of wild birds and ornamental is restricted to the Nymphs (*Nymphicus hollandicus*), canaries (*Serinus canaria*), the budgerigar (*Melopsittacus undulatus*), cockatoos (*Cacatua spp*.) and agopornis (*Agapornis spp*). These birds have a close relationship of coexistence with humans because they can relate directly with the breeder, pet store personnel and the owners at home. Holding them as pets has been associated with physical and emotional benefits, and can positively influence life quality of their owners. However, despite the benefits of living with pet birds, these can also be reservoirs of infectious agents of parasitic diseases that can be transmitted to humans. The problem lies in the lack of clinical or veterinary of these species attention, because birds have a little known microbiota. There is no report in the country, and there is a difference between the native species of the region and other reported elsewhere in the world. However, several studies report that birds are reservoirs of pathogenic microorganisms to humans such as *Salmonella*, *Candida*, *Cryptococcus*, *Chlamydia* and others. Hence, the aim of this work was to investigate the presence of enterobacteria into samples obtained of nymphs and canaries from Aviary. Two samples were taken following the guidelines of a simple randomized and selected existing population of 29 specimens in the first sample and 21 in the second, taking beak, cloaca and fresh feces samples. The samples were transported and laboratory processed in different selective and differential media as Mc Conkey, Eosin-Methylene Blue, Brilliant Green Agar, Sabouraud-Dextrose and Bismuth Sulfite *Glucose Glycine Yeast medium* to isolate different microorganisms. Once isolated colonial morphology was recorded and biochemical tests were performed with the Citrate Simmons, Iron-Triple Sugar, Iron-Lysine, Motility-Indole-Ornithine and Christensen´s urea broth. The results were analyzed by ABIS online software yielded the presence of *Citrobacter spp., Escherichia spp., Serratia spp., Enterobacter spp. Hafnia spp., Yersinia spp., y Shigella spp* for both birds in most samples. These results were corroborated with Vitek compact 2, which are consistent for identification of *Escherichia coli, Citrobacter brakii, Enterobacter cloacae, Kocuria varians, Serratia marcescens, Candida albicans, Cryptococcus laurentus, Pantoea agglomerans, Streptococcus pneumoniae y Staphylococcus lentus.* In conclusion, the Nymphs and canaries have an opportunity microbiota that can be pathogenic for the guest and owner.

**Fate of the host proteins internalized by cysticerci of *Taenia crassiceps*.**

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Cysticercosis is caused by the larval stage (cysticercus) of *T. solium*. Cysticerci are vesicles filled of fluid, with a scolex, and can establish in different host tissues. Many efforts have been directed to understand the processes involved in the host-parasite relationship, which allow the long lasting permanence of cysticerci and the evasion of the host immune response. It has been known for years that cysticerci internalize intact host proteins; these host macromolecules may represent up to 13% of the protein content in the vesicular fluid. Some of the host proteins identified in the vesicular fluid are: albumin, different classes of immunoglobulins, haptoglobin, hemoglobin, several serpins and many other proteins. It has been proposed that internalization of albumin is implicated in the regulation of the parasite's osmotic pressure, whereas the internalization of host immunoglobulins, included those with antibody activity, allows the removal of potentially damaging molecules from the host-parasite interface, also constituting a mechanisms for the acquisition of nutrients (amino acids), in view of the limited ability for the biosynthesis of amino acids evidenced by the characterization of the *T. solium* genome.

In the present work, we have produce a metabolically labeled murine IgG through the use of tritiated leucine during *in vitro* culture of a hybridoma. Immunoglobulins were then purified by affinity chromatography using Protein G. Cysticerci were maintained in vitro in a culture media supplemented with the metabolically labeled IgG, during nine days. Preliminary results showed that some parasite proteins are synthesized using the degradation products of the labeled-IgG, suggesting that uptaken host proteins were used by cysticerci as an amino acids source. We also describe a new strategy, based on mass spectrometry to identify labeled proteins.

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# Defensin γ-Thionin from *Capsicum chinense* has Immunomodulatory Effects on Bovine Mammary Epithelial Cells during *Staphylococcus aureus* Internalization

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Defensins are members of the antimicrobial peptide superfamily that are produced in various species from different kingdoms, including plants. Plant defensins exhibit primarily antifungal activities, unlike those from animals that exhibit a broad-spectrum antimicrobial action. Recently, immunomodulatory roles of mammal defensins have been observed to regulate inflammation and activate the immune system. Similar roles for plant defensins remain unknown. In addition, the regulation of the immune system by mammalian defensins has been studied in humans and mice models, particularly in immune cells, but few studies have investigated these peptides in epithelial cells, which are in intimate contact with pathogens. The aim of this work was to evaluate the effect of defensin thionin from *Capsicum chinense* on the innate immune response of bovine mammary epithelial cells (bMECs) infected with *Staphylococcus aureus*, the primary pathogen responsible for bovine mastitis, which is capable of living within bMECs. For the experiments of the present work, the defensin γ-thionin from *C. chinense* chemically synthesized (100 ng/ml) was used. Primary cultures of bMECs 24 h were treated with the peptide (24 h) in the absence or presence of *S. aureus* (2 h, ATCC-27543). qPCR assays, flow cytometry and transcription factor microarrays were performed. Our results indicate that thionin at 100 ng/ml reduced the internalization of *S. aureus* into bMECs (~50%), and modulated the innate immune response of these cells by inducing the mRNA expression (~5-fold) and membrane abundance (~3-fold) of Toll-like receptor 2 (TLR2), as well as by inducing genes coding for the pro-inflammatory cytokines TNF-α and IL-1β (~14 and 8-fold, respectively) before and after the bacterial infection. γ-thionin also induced the expression of the mRNA of anti-inflammatory cytokine IL-10 (~12-fold). Interestingly, the reduction in bacterial internalization coincides with the production of other antimicrobial products by bMECs, such as nitric oxide (NO) before infection, and the secretion to the medium of the endogenous antimicrobial peptide DEFB1 after infection. γ-thionin inhibited ERK 1/2 and p38 kinases, but in bMECs-treated with the defensin and infected with *S. aureus* ERK1/2 was activated. As consequence of this activation, the transcription factors Egr-1 and NF-1 were activated in the presence of γ-thionin, which may be associated with the stimulation of the innate immune response in bMECs. The results support the potential use of defensins from plants as immunomodulators of the mammalian innate immune response.

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# Assessment of the antitumor and immunostimulatory activity of a commercial poultry vaccine

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**Introduction**. According with the World Health Organization (WHO) cancer is the main cause of death worldwide, reaching up to 7.6 million deaths for year. In brief, cancer is the result of a decrease in the Programmed Cell Death (PCD) or an uncontrolled increase in the cell proliferation. Conventional treatments such as radio and chemotherapy are the most used; however, efficacy, accessibility and specificity are limited as well as undesirable side effects. Non-traditional therapies, such as oncolytic viruses have emerged and probe their effectiveness since the middle of last century. Newcastle disease virus (NDV), an avian virus that causes important economic losses for poultry industry, has also the capability to kill cancer cells selectively, without affecting normal cells. In this regard, our group is focus in the assessment of the antitumor and immunostimulatory activity of a commercial poultry vaccine. **Method**. NDV was clarified from a commercial vaccine by ultracentrifugation in 20% sucrose cushion and concentrated to 10,000 hemagglutination units (HU)/ml by hemagglutination assay, where one HU is the highest dilution in which virus agglutinate erythrocytes. For antitumor activity determination, NDV was incubated in three concentrations, 0 (negative control), 10, and 50HU with HeLa, HCC1954, HL-60 or HepG2 cells for 24 and 48h. After that, cytoplasm nucleosomes (PDC indicator) were measured by ELISA and optical densities (OD) were expressed as Enrichment Factor (EF), which represent the quotient of treated against negative control. For immunostimulation activity, PBMC's were isolated from healthy donors using Lymphoprep™ and cultured overnight with 0 (negative control), 10, and 20HU, and LPS (5ug/ml, positive control). Supernatants of each group were collected and analyzed by ELISA for IFN-α and TNF-α production. **Results**. All cell lines showed sensitivity to the NDV in a dose and time dependent way, showing statistic significance ($p<0.05$) according to the EF of control in all conditions tested, except with HeLa + 10HU at 24h. The best antitumor activity for HeLa, HCC1954 and HepG2 was seen with 50HU at 48h, meanwhile for HL-60 was with 50HU at 24h. On the other hand, stimulation with 10 and 20HU's on PBMC's induce the secretion of higher concentrations of IFN-α and TNF-α than negative control group. For IFN-α response was dose dependent, obtaining the highest concentration with 20HU, meanwhile TNF-α production was the same for both NDV doses. **Conclusion**. NDV clarified from a commercial vaccine demonstrated antitumor activity over the four cell lines assayed in a dose and time dependent way; and it showed to stimulate IFN-α and TNF-α production when incubated with PBMC's.

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# Telomere repeat binding factors in *Entamoeba histolytica*

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Telomeres are specialized structures at the end of chromosomes essential for maintaining genome stability and cell viability. Telomeric repeat binding factors 1 and 2 (TRF1 and TRF2) are telomeric double-stranded DNA binding proteins because of a Myb-like DNA-binding domain (DBD). These proteins regulate telomere length and protect chromosome ends. *E. histolytica* contains three genes encoding for EhTRF-like proteins with significant sequence similarity with higher eukaryotic TRFs in their c-terminal DBD Myb-like (25 to 35%) DBD, but only weak similarity in their N-terminal domains. Multiple alignment of the DBD amino acid sequence from TRF-1, TRF-2 and EhTRF-like proteins, showed that the VDLKDKWRT motive is conserved in the third alpha helix where the lysine and arginine residues are involved in the DNA recognition. In this work we determinate the effect of DNA damage by $H_2O_2$ treatment in the expression of EhTRF-like proteins. qRT-PCR analysis of trophozoites under basal conditions, and after $H_2O_2$ treatment showed that *Ehtrf-like* genes overexpress immediately after treatment but only with low concentrations of $H_2O_2$. By the other hand, we selected and over-expressed one EhTFR-like protein in *E. histolytica* trophozoites and observed its nuclear localization by western blot using subcellular fractions, indirect immunofluorescence and electron microscopy. EhTRF-like protein localize mainly in regions of more condensed chromatin, which suggest an interaction with DNA. These results provide the first evidence that EhTRF-like proteins might participate during damage to DNA protecting telomeric DNA from the instability caused by oxidative stress as telomeric proteins in *Entamoeba histolytica*.

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# Structural characterization and cellular localization of Tv-PSP1, a perchloric acid-soluble protein of *Trichomonas vaginalis.*

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**Introduction:** Perchloric acid-soluble proteins (PSP) are biologically important by participating in cell survival. Recently, our research group found three genes that encode for PSP in *Trichomonas vaginalis*, the causal agent of human trichomoniasis. An *in silico* experiment; showed that Tv-PSP1, is a member of the YER057c/YjgF family, which are homotrimeric proteins. The main goal of this study was the structural characterization of recombinant Tv-PSP1 and to determinate its cellular localization in *T. vaginalis*. **Methodology:** Biophysical techniques such as circular dichroism, size-exclusion chromatography and dynamic light scattering were used to rTv-PSP1 structural characterization. Protein stability was determined by molecular dynamic simulation. Homology modeling was used to predict the Tv-PSP1 three-dimensional structure. Tv-PSP1 cellular localization was determined by transmission microscopy electron using an anti-rTv-PSP1 polyclonal antibody. **Results**: According to structural analysis, rTv-PSP1 has an $\alpha + \beta$ class secondary structure and its 13.5-kDa monomeric structure forms a ~40.5-kDa homotrimeric protein. Furthermore, the quaternary structure of rTv-PSP1 showed high stability according to the molecular dynamics of 100 ns compared to the initial model, with a RMSD of 1.75 Å. Moreover, Tv-PSP1 is located in hydrogenosomes, nucleus and cytoplasm in *T. vaginalis*. **Conclusion:** rTv-PSP1 is a 40.5-kDa homotrimeric stable structure formed by 13.5-kDa monomers that it may be involved in several unknown biological processes in *T. vaginalis.*

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# Analysis of the adaptive immune response in mice with Chagas' disease treated with NIPOx-B

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Chagas' disease is caused by the parasite *Trypanosome cruzi*; it affects 10 million people in Latin America and it remains incurable: the only drugs available for its treatment, Benznidazole and Nifurtimox, are inefficient to cure patients. The chronic phase of the disease is characterized by megacolon and megaesophagus; this phase is established by parasite evasion of the host adaptive immunity, a process that is facilitated by the free radicals generated by Benznidazole and Nifurtimox, which affect lymphocytes and other immune cells. We designed and synthesized the benzyl ester of N-isopropyl oxamic acid (NIPOx-B) and determined that it is an effective inhibitor of alpha-hydroxy acid dehydrogenase II enzyme, a key enzyme in the parasite metabolism. NIPOx-B is more efficient than Benznidazole and Nifurtimox in decreasing mice parasitemia. In this work, we studied by flow cytometry some of the cell populations of the adaptive immune response in mice with Chagas' disease, untreated or treated with Benznidazole or with NIPOx-B. The percentage and the activation of B cells, macrophages, CD4 lymphocytes, dendritic and follicular dendritic cells in germinal centers were higher in mice treated with NIPOx-B than in mice treated with Benznidazole. The IgG and IgE antibodies titers were also higher in mice treated with NIPOx-B. Untreated mice had the lowest cell percentages and antibody titers. Mice treated with NIPOx-B did not develop megasyndromes, while untreated mice or mice treated with Benznidazole developed them. Because NIPOx-B has trypanomicidal activity and did not affect the percentage of cells involved in the immune response it can be proposed as a drug for the treatment of Chagas' disease.

# Employing cactophilic *Drosophila* species to study metabolic diseases

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The cluster of insulin resistance, dyslipidemia, visceral obesity and endothelial dysfunction is known as the metabolic syndrome (MetS), a disease of epidemic proportions and serious health problems associated [1]. Risk factors include genetic susceptibility, as ethnicity or gender, as well as ambient factors, such as diet and lifestyle. But their interaction in the development of the MetS still needs to be elucidated. To gain biochemical insights about metabolic diseases, we investigated the effect of nutritional changes on different *Drosophila* species, the fruit fly *Drosophila melanogaster* and its cactophilic relatives *Drosophiola arizonae* and *Drosophila mojavensis.* In contrast to the generalist *D. melanogaster*, the later are adapted to low carbs diets in their native environment [2]. We explored the effects of three isocaloric protein sugar ratio diets to identify biomarkers and altered metabolic pathways by mass spectrometry techniques. Employing machine-learning algorithms such as Random Forest models for data analysis revealed major effects between species. Lipids such as phosphocholines, phosphoethanolamines and ceramides differed between species. Cactophilic flies were more susceptible to changes in diet, indicating their potential use as biological models in the research of metabolic diseases.

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# *Trypanosoma cruzi*: Antigens identification of Mexican isolates.

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Chagas' disease is caused by the hemoflagellate protozoa, *Trypanosoma cruzi*. As the parasite strains are very heterogeneous, these strains have been classified into six different discrete typing units (DTU from TcI to TcVI) that present differences in the morphology of their blood forms, virulence, ability to induce injury, susceptibility to chemotherapeutic agents, antigenic constitution, infectivity and geographic distribution.

The biological and molecular variability of the different DTUs make difficult, the identification of biological markers useful for the efficient recognition of infected people with *T. cruzi*. It has been reported that the use of parasite antigens and sera of infected people, coming from different geographical origin, results in a lower levels of reactivity in comparison with antigen and sera coming from the same areas. Besides sera for infected people with TcI strains show even lower levels of reactivity. As TcI is the predominant DTU of *T. cruzi* in Mexico and doesn't exist parasite antigens of Mexican strains well identified and characterized, in this work we analyzed the antigens profile from isolates (15) with different DTUs (TcI, TcII and TcV) obtained in Oaxaca State, using sera (56) of infected individuals coming from the same areas.

Western blot analysis, using trypomastigotes protein extracts, show a different antigenic profile between strains with different DTU. Besides, the analysis using protein extracts from epimastigotes and trypomastigotes, exhibit a better recognition with protein of trypomastigotes. The identification of antigens detected by co-immunoprecipitation analysis linked to mass spectrometry assays will be presented.

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# Erythrocyte Sialoglycoproteins Bind Siglec-9 to Maintain Neutrophil Quiescence in the Bloodstream

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## Abstract

Healthy circulating blood neutrophils are functionally quiescent and exit the bloodstream after a short life span, undergoing apoptosis and macrophage-mediated clearance. Neutrophil lifespan and activation are relevant to clinical situations such as infection susceptibility, granulocyte transfusions and neutrophil-mediated inflammatory pathologies. We found that neutrophils express activation markers and progress faster to apoptosis when purified from whole blood, suggesting separation from blood factors that normally maintain quiescence. Indeed, erythrocytes suppress neutrophil activation and enhance viability. The abundant erythrocyte sialoglycoprotein Glycophorin A engages neutrophil Siglec-9, a sialic acid-binding receptor that regulates innate immune cell activation. Modifying erythrocyte sialic acid side chains eliminates Siglec-9 binding, allowing neutrophil activation. Thus, erythrocyte sialic acids have an unexpected function in regulating innate immunity and maintaining neutrophil quiescence in the bloodstream. Our findings are relevant to physiological and pathological processes involving neutrophils, and suggest reevaluation of some prior studies of activation, function and kinetics using isolated neutrophils.

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# The zinc-mediated 50 kDa-metalloproteinase, TvMP50 of *Trichomonas vaginalis* is involved in cytototoxic prostatic cells

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**Introduction**: *Trichomonas vaginalis* is a protozoan parasite with the ability to adapt to microenvironmental male urogenital tract such as the trichomonicidal $Zn^{2+}$ concentrations. This adaptation is mediated by several molecules, including proteinases. Here, we performed the function analysis of the previous identified 50-kDa metalloproteinase TvMP50, as a new virulence factor mediated by $Zn^{2+}$. **Methodology**: We used quantitative RT-PCR to analyze the mRNA expression profile of the *mp50* gene. Cytotoxicity assays were performed with parasites grown in normal and 1.6 mM $Zn^{2+}$ and monolayer prostatic cells DU145. For the inhibition assay, we used polyclonal anti-TvMP50r antibodies. By Western blot and zymograms assays, we found that native TvMP50 is secreted and it has proteolytic activity in normal and 1.6 mM $Zn^{2+}$ conditions. **Results**: The *tvmp50* mRNA levels of parasites grown in $Zn^{2+}$ presence were higher with respect to the normal conditions. Parasites grown in normal and $Zn^{2+}$ conditions were cytotoxic on the monolayer prostatic cells and the treatment of these parasites with polyclonal anti-TvMP50r antibodies and metalloproteinases inhibitors affected the cytotoxicity toward DU-145 prostatic cells. This endogenous TvMP50 is present in the secretion products of parasites grown in normal and $Zn^{2+}$ conditions. The proteolytic activity secretion of TvMP50 region was inhibited with metalloproteinases inhibitors such as EDTA, EGTA and 1,10 Phenanthroline. Conclusion: Furthermore, these data suggested that the TvMP50 metalloproteinase is a new virulence factor, modulated by $Zn^{2+}$.

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# Effect of cola consumption on growth and insulin resistance in rats with nutritional imbalance.

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The thrifty phenotype hypothesis proposes that the epidemiological associations between poor fetal and infant growth and the subsequent development of type 2 diabetes and the metabolic syndrome result from the effect of poor nutrition in early life, which produces permanent changes in glucose-insulin metabolism.[1]
The type 2 diabetes is a metabolic disorder characterized by insulin resistance and hyperglycemia. Diabetes is a disease caused by both genetic factors and environmental factors, such as the sedentary lifestyle and excessive consumption of foods rich in sugars. Consumption of sugar- sweetened beverages promotes the development of metabolic syndrome and type 2 diabetes mellitus in humans.[2]
The objective of the present work is to study the effect of consumption of cola in rats with a poor nutrition *In Utero* and during the lactancy.
Two offspring groups of male Wistar rat were studied: Control and malnourished. The mothers of the control animals had no manipulation whereas the mothers of the malnourished animals were underfed during the pregnancy and the lactancy of their offspring. The offspring of both groups were weaned at the 21 days old and they were separated in the following groups: Water (A), Coca-Cola Zero (CZ) and Coca-Cola (CC). Every animal was weighed once a week for three months. At the three months old an intraperitoneal glucose tolerance test (IGTT) was realized.
The malnourished animals had a lower gain of weight than the control animals. Within of the control group, the animals with greater weight gain were the group CC, followed by the group A and finally the group CZ. Whereas the malnourished group, the animals with the greater weight gain were the group A, followed by the group CC and finally the group CZ.
In the IGTT, the animal of the group CC had a fewer response to insulin than the other groups, both group control and malnourished.
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# Angiogenesis is controlled by miR-204 through dual targeting of pro-angiogenic ANGPT1 and TGFBR2 genes in breast cancer

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**Background**: MicroRNAs (miRNAs) have emerged as a prominent class of negative regulators of gene expression. These non-coding small RNAs function in posttranscriptional gene silencing by complementing with the 3′ UTR of target genes resulting in mRNA degradation or translational repression. Remarkably, aberrant expression of miRNAs may contribute to development of diverse neoplasia and correlates with clinical-pathological features of tumors thus representing useful prognostic markers and novel therapeutic targets in cancer.

**Results:** Studying the miRNome of breast tumors of Mexican patients, we identified 54 miRNAs (34 downregulated and 20 upregulated) in clinical specimens. Interestingly, we found a consistent repression of miR-204, a small-RNA with no previous involvement in angiogenesis in breast cancer. Gain-of-function analysis showed that restoration of miR-204 impairs cell proliferation, anchorage-independent cell growth, migration, invasion and angiogenesis. Moreover, formation of capillary tubular 3D structures indicative of vascular angiogenesis was also suppressed *in vivo*. Genomic-wide profiling of MDA-MB-231 cells expressing miR-204 revealed that 549 genes were significantly modulated. Of the 238 repressed genes a subset of 22 genes contained potential miR-204 binding sites. Of these, we demonstrated that pro-angiogenic ANGPT1 and TGFβR2 genes are novel effectors downstream of miR-204. Congruently, an inverse correlation between the miR-204 and ANGPT1 and TGFβR2 expression in breast tumors was found. Moreover, knockdown of TGFBR2, but not ANGPT1, abolish cell proliferation and migration, whereas inhibition of both genes inhibits angiogenesis. Our findings reveal a novel function of miR-204 in angiogenesis *in vitro* and *in vivo* by targeting key pro-angiogenic genes. We propose that molecular manipulation of miR-204 levels may represent a promising approach in breast cancer therapy.

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# Apoptosis-like mitochondrial membrane permeabilization in *Trypanosoma brucei.*

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Humans resist infection by the African parasite *Trypanosoma brucei* owing to the trypanolytic activity of the serum apolipoprotein L1 (APOL1). Following uptake by endocytosis in the parasite, APOL1 forms pores in endolysosomal membranes and triggers lysosome swelling. Here we show that APOL1 induces both lysosomal and mitochondrial membrane permeabilization (LMP and MMP). Trypanolysis coincides with MMP and consecutive release of the mitochondrial TbEndoG endonuclease to the nucleus. APOL1 is associated with the kinesin TbKIFC1, of which both the motor and vesicular trafficking VHS domains are required for MMP, but not for LMP. The presence of APOL1 in the mitochondrion is accompanied by mitochondrial membrane fenestration, which can be mimicked by knockdown of a mitochondrial mitofusin-like protein (TbMFNL). The BH3-like peptide of APOL1 is required for LMP, MMP and trypanolysis. Thus, trypanolysis by APOL1 is linked to apoptosis-like MMP occurring together with TbKIFC1-mediated transport of APOL1 from endolysosomal membranes to the mitochondrion.

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# Distinct phosphorylation patterns regulate $\alpha_{1D}$–adrenoceptor signaling and desensitization.

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Human $\alpha_{1D}$-adrenoceptors ($\alpha_{1D}$-ARs) are seven transmembrane-spanning proteins that mediate the actions of noradrenaline in homeostasis maintenance and physiopathology of hypertension and benign prostatic hyperplasia. Recently, there has been a growing interest in investigating how receptor intracellular domains as well as post-translational modifications, such as phosphorylation, modulate signaling. However, there are no studies focused on the structure/function of $\alpha_{1D}$-AR carboxyl tail and third intracellular loop. Therefore, the objective of this work was to identify and functionally characterize the phosphorylation sites and kinases involved in $\alpha_{1D}$-AR signaling triggering and turning off. We highlighted the involvement of receptor intracellular domains in this process. In order to achieve this, we used a combination of *in silico* analysis, mass spectrometry and site-directed mutagenesis. Then we assessed receptor phosphorylation, intracellular calcium, mitogen activated protein (MAP) kinase ERK activation and vesicular traffic.

We found a differential phosphorylation pattern upon noradrenaline receptor activation (homologous desensitization) as compared to pharmacological induced heterologous desensitization. Phosphorylation sites were found specifically at the third intracellular loop (IL3) and receptor carboxyl tail. These results were further confirmed evaluating phosphorylation of soluble IL3 and carboxyl-truncated receptors in the presence or absence of the following substitutions at the third intracellular loop: Ser 300/323/331/332/334Ala/Thr328Val. Our results suggest that $\alpha_{1D}$-AR phosphorylation residues may determine subcellular localization. Additionally, carboxyl tail Ser 486, 492, 515, 516, 518, 543 and Thr 442, 447, 507 seem to be necessary for receptor internalization and MAP kinase ERK regulation. In contrast, calcium response turning off is carboxyl tail independent. Furthermore, we experimentally demonstrated that the G protein coupled receptor kinase GRK2, phosphorylate $\alpha_{1D}$-AR upon noradrenaline stimulation, whereas PKC isoforms α/β during heterologous desensitization. Thus, GRK2 and PKC participate in generating the distinct signaling outcomes reported here.

This work represents the first $\alpha_{1D}$-AR functional phosphorylation site mapping during homologous and heterologous desensitization. Finally, knowledge of the functional domains of the $\alpha_{1D}$-adrenoceptor might help us better understand its implications in physiology and disease and could lead to the design of more effective therapeutic agents.

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# Natural variation of the mutational effects of the Tor Pathway on yeast aging

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The chronological lifespan CLS of the budding yeast *S. cerevisiae* is the time that a nondividing population of cells survive in culture and is studied to understand aging. The entire collection of singe gene knockouts has been measured to determine the effects of most deletions on aging. Some of the genes found to affect lifespan have been confirmed in a variety of species. One example is the signaling pathway of Tor, which senses and responds to nutrient conditions from yeast to mammals. Given that the genetic background may modify the magnitude and direction of the effects of mutations, assessing the effects on survivorship of deleting genes from the Tor-pathway in different strains will provide with insights on the conservation of mutational effects and the contribution of this pathway in aging. Here, we quantify the effects of deleting up to nine genes of the Tor-pathway from eight yeast strains with different genetic backgrounds isolated from diverse geographical and ecological origins. Then we measured the effects of such mutations on longevity using a high resolution method that uses flow cytometry. In brief, we count the number of RFP-tagged mutant cells and YFP-tagged wild type cells in an aging co-culture. While time passes, changes in the proportion of RFP to YFP cells reflect the relative survivorship of each mutant to the wild type. Our results show extensive variation in the phenotypic effects of deleting these genes. In order to suggest mechanisms, we are taking advantage of the large databases of both phenotypes and genotypes of *S. cerevisiae* to analyze possible correlations with our findings. Once we recognize candidate processes, we will replace some alleles in order to corroborate their interaction with the pathway. Our findings suggest that the effects of modifying this pathway to extend longevity in other organisms are different depending on the genetic background of the individuals in which the modifications are tested. It remains to be elucidated what are the underlying genetic mechanisms of differences as well as their conservation across different species.

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# Damage-associated molecular patterns (DAMPs) and signalling during mechanical injury in filamentous fungi

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Responses to mechanical injury are crucial for the survival of any multicellular organism, enabling their adaptation to hostile environments. *Trichoderma atroviride*, responds to different environmental stresses by producing asexual reproduction structures. Recently we found that *T. atroviride* responds to mechanical damage by activating regenerative processes and conidiation. During this response, reactive oxygen species (ROS) are produced by the NADPH oxidase complex.

To understand the underlying early signalling events, we evaluated molecules such as extracellular ATP (eATP) and $Ca^{2+}$ that are known to trigger wound-induced responses in plants and animals. Concretely, we investigated the activation of mitogen-activated protein kinase (MAPK) pathways by eATP, $Ca^{2+}$, and ROS. Mutants in the MAPK-encoding genes *tmk1* and *tmk3* were affected in regeneration process and wound-induced conidiation. Phosphorylation of both Tmk1 and Tmk3 was triggered by injury and eATP, in contrast calcium signaling appears to take place through a MAPK-independent pathway. Indeed, application of exogenous ATP and $Ca^{2+}$ triggered conidiation.

To verify the role of calcium in the response to wounding in other filamentous fungi, we used a calcium reporter, Calmodulin-GFP (GCamP6) in *T. atroviride* and *the pathogenic fungus Aspergillus fumigatus*. Interestingly, eATP and injury cause a transient increase in $[Ca^{2+}]_c$ .This increase depends on extracellular calcium and is likely regulated by L-type calcium channels on the plasma membrane that further activate the release of intracellular $Ca^{2+}$ pools.

These results suggest that extracellular ATP serves as a cue that signals tissue disruption toward unbroken cells and that a calcium-signaling pathway plays an important role in early wound response. Thus, the early and late steps of the injury response in filamentous fungi include mechanisms that are shared with plants and animals.

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# Regulation of SnoN expression by TGFβ and GPCR signals in Hepatocytes

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SnoN (Ski-novel Protein) was discovered as a proto-oncoprotein, with a main function as a transcriptional co-repressor of TGFβ/Smad signaling pathway. SnoN plays important roles in several processes like proliferation, differentiation and aging. The regulation of SnoN expression is very dynamic and occurs at multiple levels, including gene transcriptional regulation, mRNA translation, protein stability, and protein subcellular localization. TGFβ is the main signal that regulates the stability of SnoN protein and its gene expression.

We have reported that the SnoN mRNA and protein are increased during hepatic regeneration, induced by partial hepatectomy, by an unknown mechanism. Now, in the present study, we identify and purpose additional signals and mechanisms, distinct to the TGF-β pathway, able to control SnoN expression in hepatocytes.

At the transcriptional level, we identified a putative super-enhancer upstream of the mouse *sno* gene promoter, composed by a cluster of 8 enhancer sequences, which were defined by epigenetic marks such as H3K4me1, H3K27ac, and also by the presence of transcriptional factors as Med-1, p300 and Pol II, distributed in a region of 22 Kb.

Furthermore, we showed that SnoN protein levels are increased, when compounds as Sphingosine 1-Phosphate, CytochalasinD and Jasplakinolide induced actin polimerization, and this increase of SnoN levels was independent of *sno* gene transcription. Our data suggest that TGFβ-independent signals like GPCRs can also regulate SnoN expression in hepatocytes by modulating actin polymerization

Our work is supported by grants from Conacyt and PAPIIT/DGAPA/UNAM.

# Molecular and biological characterization of TcVps26-Like of *Trypanosoma cruzi.*

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Vps26 is a protein that it belongs to the arrestin superfamily, that has been proposed can interact with others proteins and is an important component of the retromer complex, which mediates the transport of proteins from endosomes to the trans-Golgi network. Previously we performed a functional analysis of a molecule that is differentially expressed in an intermediate form (IF), obtained during the in vitro secondary amastigogenesis of *T. cruzi*, in comparison with trypomastigotes. This molecule is annotated in the triptryDB database of the parasites as a putative protein (TcCLB.506941.210) which, an initial in silico analysis, suggested that it could correspond to Vps26-Like of *T. cruzi*. Furthermore, the obtaining and analysis of Knockdown (KD) parasites of Vps26-Like, shows a severe deleterious effect in the ability of the KD parasites to invade and infect target cells.

In order to determinate if Vps26-Like, corresponds to Vps26 of *T. cruzi*, we evaluated in KD parasites and mock control, its localization, participation in the organization of the Golgi apparatus and possible colocalization with different markers of endosomal system, where the retromer complex is a vital element of the endosomal protein sorting machinery.

A deep in silico analysis, showed that the putative protein under study, contained a pfam03643 domain, which correspond to the Vps26 proteins, and present an identity of 11.99% with Vps26A of human. Additionally, a 3D modeling in Phyre2, showed that the folding of Vps26-Like sequence has a 100% confidence with the crystal structure of Vps26B from mouse and human, with an identity of 21% and 19%, respectively. Also, TcVps26-Like showed a cytoplasmic localization mainly as spots in the posterior part with respect to the nucleus of the epimastigotes form, similarly as it has been reported for Vps26 in others eukaryotic systems. Also, the KD parasite showed a fragmentation of the Golgi apparatus in comparison with control parasites (mock), as it is characteristic in various eukaryotic systems. Finally, TcVps26-Like colocalizes with markers of early and late endosomes. All these result together, strongly suggest that Vps26-Like correspond to Vps26 of *T. cruzi*.

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# Histamine Impairs Midbrain Dopamine Neuron Differentiation in Association to Modifications of Epigenetic DNA Marks.

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During rat midbrain (MB) formation, dopamine neuron differentiation occurs between embryonic days (E) 9 to 15. During this process, the regulation of extrinsic and intrinsic signals that modulate the expression of different transcription factors at temporal and spatial levels, are fundamental. Modifications of epigenetic marks in histones and DNA are essential for the expression of genes required for dopaminergic differentiation. DNA demethylation process has an important role during neurogenesis, since increases of 5-hydroxymethylcitosine (5hmC) has been associated to transcriptional activation of neuronal genes. Recently, we demonstrated that injection of histamine (HA) in the cerebral ventricle at E12 decreases the number of mesencephalic dopamine neurons. However, the mechanism of HA´s action remains undefined. Here we show that ultrasound-guided injections of HA at E12 decrease the percentage of 5-methylcytosine (5mC) on the exons of *Pitx3* and *Th*, suggesting a role of HA on the transcriptional regulation of these genes. Using qRT-PCR, we found that HA decreases the expression of early genes involved in midbrain dopaminergic specification, such as *Lmx1a*, *Msx1* and *Foxa2* and importantly, also down-regulates the terminal differentiation genes *Pitx3* and *Th*. Thus, decreases of 5mC on *Pitx3* and *Th* on gene body, correlated to lower levels of transcripts of these late dopamine neuron differentiation genes. We also found that HA has a long-term effect on the dopaminergic axonal growth, since the administration of HA at E12 decreased the number of neuronal fibers positive to Tyrosine Hydroxylase in the nigrostriatal pathway, 6 days after the injection. These findings suggest a molecular mechanism of action of HA during the development of dopaminergic neurons.

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# Infected cells in root bean symbiotic nodules: an environment under controlled stress

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Rhizobial soil bacteria can form a symbiotic interaction with *Phaseolus vulgaris* in which the bacteria fix atmospheric nitrogen into ammonia that can be utilized by the host. This interaction results in the formation of a new root organ, the nodule, where the plant provides a suitable environment for N2 fixation by rhizobia. During the infection process the bacteria differentiates into a bacteroid form that is able to fix nitrogen. Infected cells (IC) from symbiotic determined nodules in *Phaseolus vulgaris* presents a quite interesting cellular landscape: (a) IC presents up to one hundred times more membranes than other tissues to form the peribacteroidal membranes (PBM), (b) they perform a highly increased protein synthesis to sustain the symbiotic interaction requirements, and (c) these IC have an important protein trafficking and secretion activity between the cytosol and the symbiosome space, selectively regulated by proteins present in the PBM. How the ICs deal with these conditions? Do these represent a stress?

In this study we show the nucleolar Hsp70 protein localization at IC in 20 days post inoculated nodules, the marker genes expression associated with stress responses, and a scrambled endomembrane phenotype under ER-Hsp70 gene silenced (BiP-RNAi) transgenic roots. These evidences strongly support the activity of stress responses in bean cells infected by rhizobia as well as the important Hsp70 role to sustain nitrogen-fixing activity in nodule IC.

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# Predicting multifunctional polypeptides using machine learning algorithms

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Many proteins in nature are cataloged as multifunctional, that is, these are endowed to perform more than one function by either combining different domains each with different function, or having multiple activities in one domain such as in the case of moonlight proteins. In this last case, it is still unknown how proteins acquire this trait. A consequence of this limited understanding about the mechanisms involved in the evolution of multifunctional proteins is that the best predictors of protein functions fail to correctly identify the multiple functions of multifunctional proteins. Machine learning algorithms have clearly improved approaches based on sequence alignments, yet even these approaches fail to properly identify multiple functions of multifunctional proteins. The main limitations of any approach to predict protein function are: i) few proteins have been experimentally determined to be multifunctional and ii) most predictors are trained to treat the structure-function relationship of proteins as a bijection (1). One way to overcome these limitations is by finding compatible activities, that is, protein functions that share the same protein sequence attributes used to train a classifier. In this way, it is possible then to label proteins with multiple compatible functions. We have experimentally shown that polypeptide sequences predicted to have compatible functions indeed are multifunctional (2). In this work, we use a computational method to predict a set of functions that can be compatible within a polypeptide. This method is based on a machine learning algorithm, which is trained to predict for a particular protein function on a set of polypeptides. For this work, the algorithm was trained to predict antimicrobial activity on peptides, with an accuracy of 90%. The predictor was tested on sequences from the PFAM database, and observed that 9891 of the 16035 PFAM families were predicted as antimicrobial. Nevertheless, most of the proteins from these families are not annotated as antimicrobial; instead, they are reported with different activities, including nucleotide binding or cellular localization (3). We have previously shown that antimicrobial peptide activity is compatible with cell penetrating (4) and DNA binding activities (2), so these results provide further support to the idea that one way for proteins to display multiple functions is by recognizing that different protein functions may in fact be obtained using the same set of protein sequence attributes. We propose this method as a way to associate multiple functions to proteins, hence providing the scientific community a testable hypothesis to identify multifunctional proteins.

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# Finishing and validation of a lager beer yeast genome sequence using a BAC-based physical map, an hybrid approach

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The most widely distributed beer in this industry is the *lager* style. This beer is produced by *Saccharomyces pastorianus*, a natural hybrid between *Saccharomyces cerevisiae* and *Saccharomyces eubayanus*. The above means that this yeast possesses copies of both parental chromosomes. Next-generation sequencing technologies allowed the retrieval of the genome sequence of a lager beer yeast strain; however, no efforts to asses and improve the quality of the genome sequences assembly were made. To this end, we sought to incorporate alternative information. One way to generate a great amount of experimental evidence are physical maps. Physical maps are representations of the DNA sequence order. We constructed a BAC-based physical map comprised of 2,304 clones and we integrated it to the genome assembly through the BAC-end sequences (BES). The physical map and the BES allowed not only to detect misassemblies and to determinate the order of the scaffolds but also to identify sequences overlaps between them. Many of the genome sequence scaffolds were consensus of different parental homologous chromosomes. Therefore, the sequence cloning strategy was advantageous compared to the whole genome shotgun assembly because it allowed us to obtain information from individual DNA molecules. For that reason, we chose selected BAC clones as templates for the gap closing of the previous determined scaffolds order. This process along with the electrokaryotypes and ploidy assessment are the final steps needed to have a finished lager beer genome sequence.

Key words: lager beer yeast, Next-generation sequencing, genome assembly, Physical mapping.

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# Inter-chromosomal transcriptional regulation in breast cancer

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Breast cancer is a complex and highly heterogeneous disease. Despite this heterogeneity, some characteristics among the different subtypes of breast cancer are shared. However, the intricate relationships that govern the regulatory program during the development of this neoplasia still remain largely unknown.

In this work, by using 819 genome-wide RNASeq breast cancer as well as 101 healthy adjacent breast tissue samples, obtained from The Cancer Genome Atlas (TCGA), we perform a network-based approach to unveil common shared features for the transcriptional regulatory program in breast cancer. A pathway-based analysis allows us to observe upregulation in processes related to cell cycle and cell division, meanwhile migration, adhesion and cell-to-cell communication result downregulated in the cancerous phenotype.

We find the *Role of BRCA1 in DNA damage* and the *Estrogen-mediated G1/S phase entry* pathways being upregulated. A synergistic underexpression of protocadherins-gamma complex is observed; this complex is located at Chr5q32 and regulates focal adhesion and migration. This region has been encountered hypermethylated in breast cancer. We observe dramatic differences in the cancer network (CN) architecture compared to the healthy network (HN): in the HN we have a giant component meanwhile the CN contains several small components (Fig 1). Furthermore, the inter-chromosomal relationships between genes in the cancer network get lost (Fig 1B), but this inter-chromosomal regulation is recovered in the healthy network (Fig 1A). We hypothesize this loosing of long-range regulation could be a novel hallmark of breast cancer. With the approach presented here we are able to unveil features that may be involved in shaping this complex disease.

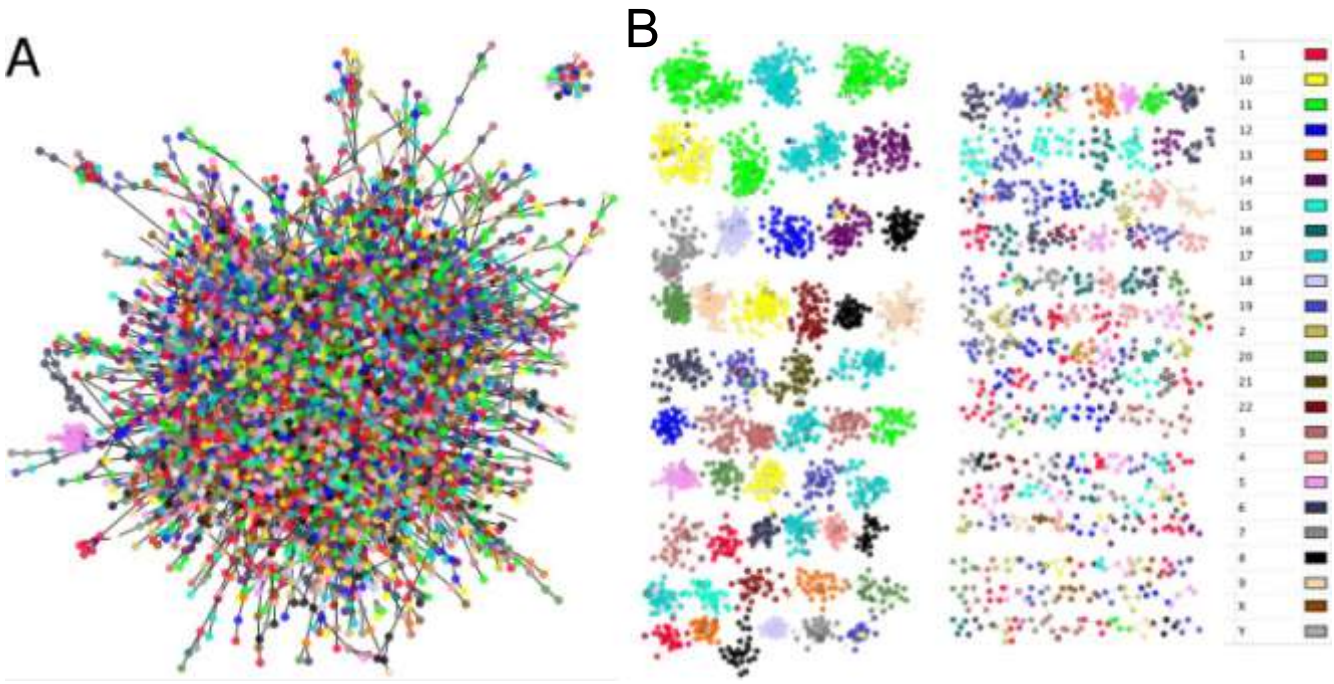


Figure 1: Healthy and cancer networks and loosing of inter-chromosomal regulation. This figure shows the architectural features of each network. A) Healthy network (HN) B) Cancer network (CN). Nodes represent genes in the network and links are correlations between each pair of nodes. The color code is according to the chromosome location in which each gene is placed. Notice the size of the giant component in HN, which is not the case for CN, where several small components coexist. Furthermore, in the HN all genes in the giant component belong to different chromosomes, meanwhile in the N (B), almost all subnetworks are composed by genes which belong to the same chromosome.

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# Visualization of F-actin in *Trichoderma atroviride* during growth and mechanical injury

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Actin cytoskeleton is essential for many important processes in all eukaryotic cells. Actin is organized in different functional structures depending on the associated Actin Binding Proteins (ABPs). In filamentous fungi, the actin cytoskeleton is involved in vesicles transport toward zones of polarized growth using actin cables, septal formation using contractile rings and endocytosis using patches. Also it has been shown that actin has an important role in cell membrane repair after injury in different eukaryotic organisms. In this work we visualized the organization and dynamics of the different actin structures and during mechanical injury recovery in mature hyphae of *Trichoderma atroviride* tagged with the reporter Lifeact-GFP. By confocal microscopy we observed F-actin associated to the core of Spitzenkörper. We also found a subapical collar of actin patches that is present after the apical dome. Some actin patches were present in older areas of the hyphae. During septal formation an actin tangle (SAT) was observed several minutes before any sign of plasma membrane invagination, the tangle coalesced forming a contractile ring that was constricted to form the septum after the mycelium of *T. atroviride* was injured with a scalpel and F-actin accumulated at the septum closest to the site of injury. Patches but not filaments were present in the apical dome until a new and thinner hypha grew from the septum. A few microns from the septum we observed the formation of a SAT and later on a CAR during the recovery process. Hyphae that did not produce these structures were not able to recover and growth. The recovery of the F-actin associated to the Spitzenkörper seems to take several hours. We conclude that all of the actin arrangements described in other organisms are present in *T. atroviride*. Actin seems to play an important role for the recovery of a new growth site after injury.

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# Pore-forming mechanism of the antimicrobial peptide Pandinin-2.

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Over the last decade several disease-causing microbes have become resistant to antibiotic drug therapy and came to be an important public health problem. Therefore there is an urgent need to develop new antimicrobial drugs in order to surmount the increasing resistance of pathogens to existing antibiotics. Naturally occurring antibiotics are a good place to start. A wide variety of organisms produce antimicrobial peptides (AMPs) as part of their defense system against pathogens. Most AMPs interact with lipid membranes causing cell lysis. The action of AMPs is a competition of the hydrophobic interactions between the side chains of the peptides and the hydrophobic region of lipid molecules, as well as the intra peptide interaction. In recent years AMPs have been considered as potential antibiotics since they act mainly against bacteria and fungi. Pandinin-2 is an amphipathic pore forming antimicrobial peptide isolated from the venom of the African scorpion *Pandinus imperator*. This peptide exhibits activity against bacteria as well as a strong hemolytic activity. Molecular dynamics (MD) simulations have been performed to analyze the structure, dynamics and pore-forming mechanism of Pandinin-2. Two membrane models, POPC and POPG, were used to understand how the phospholipid differences affect the conformation and mechanism of Pandinin-2 upon these membranes. The results indicate that the peptides are electrostatically attracted to the two types of membrane. When Pandinin-2 lies on the surface of the membrane layer it adopts a disordered structure which starts at the $COO^-$ and $NH3^+$ ends while the central residues retain their secondary structure. After 500ns of simulation, Pandinin-2 shows a toroidal pore in both membrane models.

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# Molecular signatures of mitochondrial metabolism shift in glioblastoma cell lines defined by principal component analysis.

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Cancer disease is a multifactorial biological phenomena, however shares changes on specific molecular pathways (1). One of these changes is about energetic metabolism known as Warburg effect, where glycolitic metabolism is enhanced instead of oxidative phosphorylation (OXPHOS) supposing a mitochondrial dysfunction. Although metabolic shift is a fact in many cancers, more recent evidence shows a redirection of mitochondrial function more than a dysfunction (2).
Glioma proteomics in biology is currently one of the principal research areas in cancer of central nervous system. Although many proteins have been identified their biological relevance missed out of nature´s background disease, since proteomic studies in gliomas are characterized by lists of proteins selected on basis a constrained statistical tools leaving a poor image analysis (3).
Here, in order to analyze the metabolic change, we use human T98G and U87 glioblastoma cells which present an oxidative and glycolytic metabolism respectively; both cells may also represent an initial and advanced state of disease. On previous experiments we found that OXPHOS supercomplexes are diminished in U87 cells but their roll in glioblastoma carcinogenesis is unknown. Here we analyze mitochondrial proteome in order to determine metabolic changes and other mitochondrial pathway changes.
Aim: to determine the mitochondrial proteome and define groups (modules) of relevant proteins for Warburg effect and glioblastoma carcinogenesis.
Methods: to determine mitochondrial change function, mitochondrial protein extracts were analyzed by 2D electrophoresis (IEF/SDS PAGE), image analysis were performed with PdQuest software. To assess protein expression and discriminate between involved pathways a statistical method was implemented by simple random sampling of spots in gel, data matrix imputation. Protein expression comparation was done by Principal Components Analysis (PCA).
Results: Proteomic analysis was done on 8 gels, 4 of each cell line. 356 spots were randomly selected regardless of the intensity neither the difference in expression. The database shown 18% of missing data, which were imputed by multivariate imputation methods. The PCA defined a structure with 5 components. The first one renders a clear spot pattern differentially expressed, also allowed to discriminate between cell lines and defines specific molecular signatures.
Conclusions: This procedure is more appropriate to describe the mitochondrial proteome of glioblastoma cell lines based on groups of protein expression and define molecular signatures, which clearly indicates two different metabolic systems.
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# Towards the Inference of the Atomic Three-Dimensional Structural Models of Proteins Without Crystallography nor Resonance

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Determination of the atomic three-dimensional (3D) structure of proteins has rested mainly on physical approaches such as crystallography (*e.g.*, X-ray crystallography, cryo-electron crystallography) or nuclear magnetic resonance until now. Such techniques require purified proteins that in many cases are difficult or impossible to obtain, especially in the case of transmembrane proteins. Furthermore, due to theoretical intrinsic limitations of these techniques, the solution of a 3D model of proteins commonly requires the use of theoretical models to fit the experimental data generated by these techniques. Aside from errors derived from such fitting, the 3D structures provided by these techniques may contain errors that require further experimental validation commonly derived from mutagenesis and biochemical characterization of the protein of interest.

Recent advances on protein structure prediction methods and high-throughput sequencing techniques have promoted the emergence of a new field that may provide an alternative protocol to infer the 3D structure of proteins, thus complementing the work so far done by physical approaches. In this work, I introduce different techniques developed in our group that enable us to conduct the first experiment in México to infer the 3D structure of a protein without crystallography or resonance. Our method combines experimental mutagenesis of a protein with computational approaches to generate a 3D model of a protein that is consistent with the mutagenesis data. For that end, in our group we have implemented saturation mutagenesis techniques and deep sequencing to identify the DNA coding for proteins with wild type and mutant phenotypes; the aim of such mutagenesis and deep sequencing is to identify both single and multiple mutations that will be used to guide the model construction and validate it. For that end, we have developed original machine-learning approaches for fold recognition and secondary structure prediction and a physics-based algorithm to fit a 3D atomic model of the protein sequence to the space of the predicted protein fold based on the restriction derived from multiple compensatory mutations. The generated model is further evaluated using centrality measures to test for the agreement between the experimentally determined single critical residues with those predicted by centrality. The final model generated using such procedure would not only provide with the atomic 3D structure of the protein, but with its full experimental validation based on single and multiple compensatory mutations. This procedure is subject to automate both the experimental and computational methodologies.

# Role of miRNAs in breast cancer regulatory networks

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MiRNAs are small non coding RNAs with a mature sequence of 22 nucleotides. They are associated with the transcriptional regulation of many different cellular processes, even in mechanisms related to cancer development and progression.
Breast cancer is the most frequent cancer among women and the second most common cancer in the world; as a major public health problem there have been major breakthroughs in breast cancer biology over the last years. And even though it is well accepted that the transcriptional alterations are one of the features of this disease, the miRNA role in its regulation is still unclear. In order to understand the transcriptional burden in breast cancer we constructed a miRNA-gene regulatory network.
To study the miRNA and gene expression relationships involved in breast cancer we selected 86 patients from The Cancer Genome Atlas (TCGA) database, for whom TCGA has available RNA sequencing expression data and matched miRNA sequencing expression data from invasive breast cancer and adjacent tissue. From the expression data we constructed Mutual Information (MI) based networks with mature miRNAs and mRNA interactions for cases and controls.
We found that the MI distributions for cases and controls are different, with cases obtaining consistently lower values. Because these interactions are related to the statistical dependency between expression profiles we can infer that the miRNAs' regulative programmes between other miRNAs and genes associated to each phenotype are also different.
Almost all the nodes and interactions form part of a main connected component that is represented for each condition in Figure 1. In these networks we found that the most connected nodes are different between cases and controls. To examine the biological significance of these nodes we decided to study the miRNAs as families, and found that in the network these structurally related nodes drive most of the interactions for each phenotype. The most connected miRNA family for cases is mir-199 (mir-199a, mir-199b), and mir-200 for controls (mir-200a, mir-200b, mir-200c, mir-141, mir-429). We constructed for these miRNA family members a first neighbours network of cases and controls and found that most of the miRNAs present are associated with Epithelial-Mesenchymal transition and acquisition of stem-like properties. The networks included genes related to these processes such as: ZEB1/2, TWIST1/2, SNAI2 and TGFBR2.
In the cases network we found that a considerable number of miRNA mapped to the DLK1-DIO3 region in Chromosome 14q32. MiRNAs in this region are dysregulated in many pathological processes and various types of cancer. Although this region function is not completely understood, it is known to be maternally imprinted, suggesting that it might be important for breast cancer pathogenesis and/or progression.

We conclude that the networks for cases and controls, although constructed with samples from the same patients, show structural differences related to the differences in their expression patterns, reflecting the transcriptional changes that breast tissue undergoes in becoming a tumour primary site.

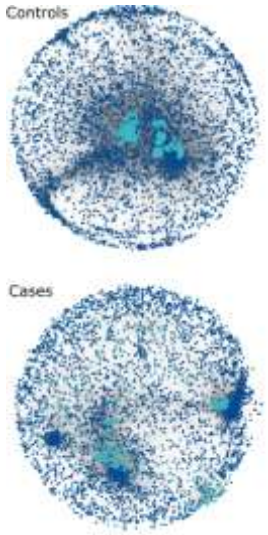


Figure 1: Largest component for controls (top) and cases (bottom networks. The turquoise nodes represent the miRNAs, meanwhile the blue nodes represent genes. The size of the nodes is proportional to the node degree. The intensity of the interaction colour corresponds to the MI value, where larger values are darker.

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# miRNOME Landscape Analysis Reveals a 30 miRNA Core in Retinoblastoma

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miRNA studies on retinoblastoma (Rb) are limited to specific miRNAs previously reported in other tumors or to medium density arrays. Because miRNAs exert their effect through a negative regulatory mechanism silencing expression upon hibridizing to their target mRNA, have a prominent position in the control of many cellular processes including carcinogenesis. In this work we examined with a broad perspective the whole miRNome expression using a high throughput microarray platform including 2578 mature miRNAs in 12 samples of primary human Rb. This is the first work to delineate the miRNA landscape in human retinoblastoma samples with a non-biased approach. We discovered a core-cluster of 30 miRNAs highly expressed in all the cases, a cluster of 993 absent also in all cases and 1022 variably present in the samples accounting for inter tumor heterogeneity. We explored mRNA targets, pathways and biological processes affected by some of these miRNAs, from this exploration we propose that the 30 miRs core represent a shared miRNA machinery in Rb affecting most pathways considered hallmarks of cancer. Interestingly, 36 miRNAs were differentially expressed between males and females, some of their potential pathways were associated with hormones. Through this exploration we also identified miR-3613 as a potential central down regulator hub, because is highly expressed by all the samples and have at least 48 tumor suppressor genes as potential mRNA targets including the RB1 gene itself. Our results indicate that human Rbs share a common and fundamental miRNA expression profile regardless of heterogeneity. The therapeutic implication of targeting mir-3613 as a potential treatment for RB patients remains to be studied.

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# Genomic reconstruction of the evolutionary history of *Phaseolus vulgaris*: from early speciation to recent domestication in America.

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The agronomic importance of common bean (*Phaseolus vulgaris*) and its pivotal role as major a dietary component in developing countries throughout the world have placed this legume as a target for genomic analyses that aim at defining the loci and associated polymorphisms behind the emergence of domestication and improvement traits. However, little emphasis has been given to the role of genetic flow between common bean subpopulations that, in other crops, has been shown to be crucial for the adaptation of cultivars to different environmental conditions.

The availability of two sequenced *P. vulgaris* genomes of Mesoamerican[1] and Andean[2] origin set the framework for deeper analyses on the genomic and population dynamics that have shaped the genomes of wild and domesticated populations we observe nowadays. In this study, we constructed an evolutionary model of common bean speciation and domestication histories by re-sequencing ten *P. vulgaris* accessions from Mesoamerica (MA) and three from the Andes (AN), together with five genotypes from the Peruvian-Ecuadorian area enclosed in the Amotape-Huancabamba deflection (AH) in the Andes, and eleven Mesoamerican Phaseolus species from the Vulgaris, Filiformis, Lunatus, Leptostachyus, Polystachios and Tuerckheimii groups. Moreover, by exploring the patterns of genomic flow, we identified signals of unbalanced inter- and intra-species genomic introgression in Mesoamerica and across American Northern and Southern hemispheres. Our screenings unveiled the capacity of a preferentially autogamous plant to outcross and fix loci from different populations even from more distant species, evidencing the mobility of stress response genes as adaptive traits. Finally, the combination of genomic, phylogenetic and metabolomics signals, allowed us to postulate a speciation event in the AH region that predates the split of the Mesoamerican and Andean gene pools, giving rise to a separate *Phaseolus* lineage that should be considered a cryptic sister species of *P. vulgaris*.

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# Computational modeling of TLR5 and TCR cooperation for adult and neonatal CD4 T cell activation.

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Toll-Like Receptor 5 (TLR5) recognizes the flagellin monomer, a component of the flagella of many bacteria. Flagellin is being evaluated as a vaccine adjuvant given its ability to induce pro-inflammatory signaling cascades in a variety of cell types. In T cells, flagellin directly provides a co-stimulatory signal to the T cell receptor-mediated (TCR) signals leading to proliferation and IFNg production. Neonatal cells are particularly poor responders to classical TCR/CD28 signals, and produce low amounts of IFNg. This study aimed to model the cross-talk between TLR5 and TCR signaling pathways leading to adult and neonatal CD4 T cell activation. We used the software GINsim to generate and analyze the logical models. First, we constructed distinct logical models for TCR and TLR5 signaling pathways based on published information and high-throughput data. Next, we validated these models using experimental data obtained in our lab. Then, we reduced these models and merged the reduced versions to obtain a model accounting for the cross-talk between the two pathways. We perform a dynamical analysis of these different models to delineate the specific effects of the cross-talk between TLR5 and TCR pathways on CD4 T cell activation. We then stimulated highly purified naïve CD4 T cells by cross-linking the CD3 molecule, in the presence or absence of flagellin, and evaluated the activation of IKKαβ, c-JUN and CREB by flow cytometry. Experimental data was used to further improve our merged model. The resulting model provides novel insights in the effects of flagellin co-stimulatory signals on adult CD4 T cell activation. Also, the experimental results show that TLR5 co-stimulatory signals are as effective as CD28 signals to promote activation of the main transcription factors. We are currently evaluating the same signalling events in neonatal CD4 T cells, and the RNA-seq of adult and neonatal CD4 T cells in basal and stimulated conditions (TCR/TLR5 and TCR/CD28). This data will be used to extend and fine-tune our model in order to obtain insights into the mechanism of neonatal CD4 T cell activation by TCR/CD28 signals and to explore the role of TLR5 pathway in neonatal CD4 T cell activation.

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# Early evolution of gene regulatory elements in the animal kingdom

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The gene regulatory systems that control animal development must have emerged alongside with the animal kingdom. The most ancient animals (sponges, ctenophores and placozoans) possess most of the known families of metazoan (animal) transcription factors. However, the DNA regulatory elements bound by these transcription factors as well as their mRNA splicing patterns remain unknown. Using bioinformatics methods and next generation sequencing data across development, we have characterized the evolution of regulatory elements and the transcriptional repertoire in a species of one of the most ancient metazoans, the marine sponge *Amphimedon queenslandica*. Sponges diverged from the rest of the animals over 600 million years ago.

Similar to its unicellular ancestors, we found that *Amphimedon*'s genes are tightly packed and have small introns, while its transcripts display low rates of exon skipping and high rates of intron retention. On the other hand and similar to observations made in bilaterians, poly A signals (PAS) are underrepresented downstream of the transcription start site (TSS), while splicing signals (5'SS) are enriched. The opposite pattern is observed upstream of the TSS. This is consistent with the production of bidirectional transcripts in unidirectional promoters, one of which is rapidly degraded after being generated. We also find evidence of promoter motifs of animal transcription factors such as Krüppel-like factors and Nrf-1. The presence of splicing patterns with high levels of intron-retention, together with the high gene density in this sponge suggests a genomic organization similar to that of unicellular ophistokonts. In contrast, the regulatory sequences in its promoters and signatures of bidirectional expression suggest these gene regulatory methods, characteristic of metazoans, emerged concurrently with the emergence of animals.

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# MicroRNA-204 impairs angiogenesis by targeting pro-angiogenic ANGPTL2 and CREB5 transcription factor in breast cancer

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**Background:** Angiogenesis is essential for tumor growth and metastasis, thus controlling tumor-associated angiogenesis is a promising tactic in limiting cancer progression. The formation of new blood vessels through the process of angiogenesis is critical in vascular development and homeostasis. However, aberrant angiogenesis leads to a variety of diseases, such as ischemia and multiple types of cancer. Recent studies have revealed important roles for microRNAs in regulating endothelial cell function, especially tumor angiogenesis. Angiopoietin-like protein 2 (ANGPTL2) is a pro-angiogenic factor that is upregulated in various types of cancer and function as an oncogene in tumor progression. On the other hand, cAMP responsive element binding protein 5 (CREB5) is associated with cell proliferation and has been described as a key protein involved in metastasis in cancer. In a previous study using genomic approaches, we identified ANGPTL2 and CREB5 as potential targets of miR-204 in metastatic triple negative MDA-MB-231 breast cancer cells.

**Results:** In the present study we evaluated if miR-204 targets ANGPTL2 and CREB5 genes in breast cancer cell lines and tumor tissues. Interestingly, using luciferase reporter gene assay, we demonstrated that restoration of miR-204 in MDA-MB-231 cells resulted in dramatically suppression of ANGPTL2 and CREB5 expression by direct binding to the 3´untranslated region (3´UTR) of both genes. Western blots assay confirmed these findings. The expression of both ANGPTL2 and CREB5 was significantly decreased after miR-204 transfection in breast cancer cells. Congruently, the expression of ANGPTL2 and CREB5 proteins was found increased in miR-204-deficient breast tumors. Finally, we found that ectopic expression of miR-204 in MDA MB-231 cells significantly inhibited the formation of 3D tubular structures indicative of classical vascular angiogenesis *in vitro*. Taken all together these results show that miR-204 controls angiogenesis by inhibition of multiple pro-angiogenic factors such as ANGPTL2 and CREB5. Outstandingly, our findings point out that these factors can be used as therapeutic targets against metastatic development by blocking angiogenesis in breast cancer cells. We propose that manipulation of miR-204 levels using angiomiRs in the settings of pathological vascularization represents a novel potential approach in breast cancer therapy.

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# Structure of the bacterial ATP synthase

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ABSTRACT

The ATP synthase is a complex molecular machine consisting of two motors linked by a rotor.One motor generates rotation by consuming energy of the transmembrane protonmotive force (Δp) derived from oxidative metabolism or photosynthesis; the other uses energy transmitted by the rotor to lead to the chemical synthesis of ATP molecules from ADP and phosphate.

This splendid molecular turbine could be separated in two domains; 1. The $F_1$, consisting of subunits α, β, γ, δ, ε and ζ in bacteria. The latest is the ATPase inhibitor[1] exclusive from the ATP synthase of the α-proteobacteria group[2]; and 2. The $F_O$, formed by subunits *a*, *b*, *b'* and the ring of subunits *c* in bacteria. The mechanism of ATP hydrolysis mediated by the $F_1$ domainat atomic level is very well known. However, the molecular details of how the Δp is used by the $F_O$ domain to produce the rotation movement remains a mystery.

In order to understand how both of this motors are coupled together and how the harvesting of the Δp by the $F_O$ domain occurs, a detailed structural analysis is required. We have crystallized the ATP synthase from *Paracoccus denitrificans*[3], this enzyme is practically unidirectional towards the ATP synthesis activity and the inhibition of its ATP hydrolysis activity involves the ζ subunit.

The structure obtained from the diffraction of this crystals at 4 Å resolution, shows new features such as the detailed position of the*P. denitrificans* ATPase inhibitor protein(ζ subunit) and its interaction with the α and β subunits. Also, the arrange of the peripheral stalk; theparallel coiled-coil architecture of the *b* and *b'* subunits and its interaction with the α and δ subunits. Additionally, the structure shows clearly the nucleotide content on the catalytic and non catalytic sites. Finally, the last attribute present in the structure, and the most elusive until now, the horizontal array of the *a* subunit that led us to a better understanding of how the proton gradient is harvested by the enzyme to produce ATP[4].

The mechanistic characteristicsobserved from this bacterial machine apply to similar molecular machines found in all living organisms. In the future work we will structurally analyze the ATP synthase with mutations of the *a* subunit present in patients with chronic degenerative deseases.

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# Annotation, phylogeny and quantitative RNA sequencing of the bean AUTOPHAGY family genes in mycorrhizal symbiosis

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## ABSTRACT

Autophagy, a process of recycling cytoplasmic constituents to generate macromolecular building blocks and energy under stress conditions, to eliminate nonessential and damaged organelles to adapt, changing nutrient conditions and maintain cellular homeostasis. Further, autophagy also plays a crucial role in cyto-protection by preventing the accumulation of toxic proteins. Recently, findings in *Arabidiopsis* show this conserved machinery of core autophagy-related (ATG) proteins has been communicating with other cellular pathways such as the ubiquitin-proteasome system, defense-signaling pathways, protein secretory pathway, and endocytic pathway. However, knowledge on legume ATG gene families and their participation during symbiosis are limited. Herein, we explored to identify and characterize the common bean (*Phaseolus vulgaris*) ATG family genes. We found fifty autophagy-related genes in the common bean genome and 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. In different common bean tissues and organs these genes were variably expressed. The quantitative expression profiles by RNA sequencing identifies, totally 27 autophagy-related genes that respond to arbuscular mycorrhizal symbiosis during active phosphate uptake stage i.e., 3 weeks post inoculation; of these, 15 genes were upregulated and 12 were downregulated relative to the uninoculated control roots. Based on the significant expression we intend 4 potential candidate genes. The analysis presented here constitutes a starting point to understand the regulation of ATGs during mycorrhizal symbiosis, however, further analysis to decipher its functional roles are yet be revealed. This work is supported by PAPIIT (DGAPA-UNAM) grant no. IA205115 to MK.A. and partially by CONACYT-240614 to M.L.

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# Global expression profiling reveals genetic networks associated with growth and development in TOR downregulated *Phaseolus vulgaris* roots

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## ABSTRACT

The **T**arget **O**f **R**apamycin (TOR) is a master regulator and highly conserved protein kinase across eukaryotes. TOR regulates metabolism, growth and life span in yeast, animals and plants in coordination with nutrient status and environmental conditions. Recent breakthroughs made possible by integrating chemical, genetic, and genomic analyses have greatly increased our understanding of the molecular functions and dynamic regulation of the *Arabidopsis* TOR kinase in photosynthetic plants. Yet, TOR regulated genetic elements of growth and development in other plants is still limited. Herein, we performed global trascriptomics profiling using Ion Torrent Next-Generation Sequencing for TOR downregulated roots of crop legume, *Phaseolus vulgaris*. Using the RNA interference (RNAi) we downregulated the *PvTOR* transcripts by 80% and were subjected to RNAseq along with appropriate controls. These analyses identified totally 6752 differentially expressed genes (fold change >2) in TOR-RNAi roots of these, 4689 genes were upregulated and 2063 were downregulated relative to the control roots. Validation of the RNA-seq data by RT-qPCR confirms the authenticity of the expression quantities. Based on gene ontology (GO) the TOR-RNAi samples show majority of the GO annotations for biological process followed by molecular function and cellular component. Further, we also provide the expression profiles for the genes that regulate growth and development in *P. vulgaris*. The data generated in this study will help understand the differentially expressed *P. vulgaris* genes regulated by master gene TOR. This work is supported by PAPIIT (DGAPA-UNAM) grant no. IA205115 to MK.A. and partially by CONACYT-240614 to M.L.

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# Evolutionary dynamics of microRNA regulatory networks

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Evolution affects individual proteins, genes and regulatory elements. All of these have interactions and evolutionary processes also act on the resulting networks. Gene regulatory networks are key for the evolution of phenotypic traits, but the evolution of these networks is not well understood [1]. MicroRNAs are small non-coding RNA molecules that regulate gene expression at post-transcriptional level. To gain more information about the process of network evolution we focused on microRNA regulatory networks. We want to compare how the connectivity of these microRNA networks changes in organisms under different selective pressures, initially focusing on primate evolution for the vast amount of available genomic resources. In their origin, primates suffered an expansion of microRNAs families and some studies have proposed that this expansion is connected with the evolution of certain phenotypic traits [2]. We used a set of microRNAs families that are conserved across mammals, predicted their respective targets and constructed the microRNA regulatory networks. We measured the rewiring rate of these networks, counting the changes of target sites normalized by network size. We did this for each species and compared them with a control of randomized microRNA sequences. Our results suggest that the rewiring rate for individual microRNA target sites is slower than for control sequences. But, when we measured rewiring of target genes instead of sites, the rewiring rate is faster than for control sequences. This could be explained if many of the rewiring events fall into genes that are being regulated by multiple sites for the same microRNA family. We now want to apply this approach to cichlid fishes because their high variation in microRNA binding sites has suggested their role as important regulatory elements in phenotypic evolution.

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# Genomic analysis of bacterial DNA methylation and repair systems

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The wide availability of genomes, biochemical and molecular information facilitate large-scale comparative studies of biological systems. This project focuses on understanding the function and evolution of the DNA methylation-guided mismatch (MMR) and very short patch repair (VSP) systems from a multi-genomic perspective. The VSP system removes GT mismatches created by spontaneous deamination of 5-methylcytosine to thymine. On the other hand the MMR system corrects errors that occur during chromosome replication [1]. MMR and VSP systems are of great importance to reduce DNA error rates in living organisms [2], but have mostly been studied focusing on individual genomes. Our comparative approach seeks to reveal the impact of these repair systems on bacterial genome evolution. The main goals of this project are:

a) Evaluate the distribution of the methylation/repair components across different taxonomic groups.
b) Develop statistical measures to test the observed against expected frequency of DNA motifs associated with methylation/repair systems.
c) Determine whether there is a correlation between the presence of the components across different genomes and the frequency of the DNA motifs.
d) Search for over- and under-representation of these motifs within genes, and see if they are related to particular biological functions.

Our preliminary results show that there is a correlation between the phylogenetic distributions of the components of the repair systems, indicative of their co-evolution. Additional information derived from our objectives will contribute to a better understanding of repair systems, and may favor the development of application of these systems in directed evolution experiments.

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# Revealing metagenomic microbial diversity using reference pan-genomes

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Bacterial genomes are known to have a high level of variability between species in terms of gene content. Comparative genomics of bacteria and its large within group gene content variation have aroused the concept of the pan-genome, which is the complete repertoire of genes within a species. The pan-genome can be divided into a core genome, containing all the common shared genes within the species and the accessory genome where strain specific genes can be found. The operational definition of a bacterial species is a group of individuals with more than 97% sequence identity on the 16S rRNA gene and a 70% genome hybridization. Genome comparison of related bacteria strains may aid us to make insights into taxonomic problems, such as the aforementioned species definition by taking into account the complete genomic information. The whole genome information can be useful for the identification of species or strains within complex environments, such as a metagenomic samples.

In this work, we compared 108 *Streptoccus* sp. genomes and their predicted proteomes. The Streptococci are diverse and comprehends a large number of important human and animal pathogens as well as some commensal species all the diverse Streptococci were compared in order to find out their shared features (core genome), and the whole group coding gene sequences repertoire, its pan-genome. To calculate the core genome gene composition we made a search of the shared orthologs by pairs of genomes by means of Bidirectional Best Blast Hit strategy. With the pan-genome we calculated protein families using the the 108 predicted proteomes with the CD-HIT algorithm. The core genus genome is composed by only 404 proteins which is astoundingly low number of shared common features across the Strepococci, while the pan-genome is composed of 25,701 protein families. All the pairwise proteome orthologs comparisons amongst the Streptococci were used to calculate a Genomic Similarity Score (GSS), this information was used build pairwise distance matrix in order to plot a genus' dendrogram which was compared to a classic 16S rRNA gene phylogeny. The average identity of the core genes in each species showed to decrease as the GSS distance between them increased, showing GSS's promising results to discriminate amongst closely related species. Finally, samples from human oral metagenomes were used to identify species by the recruitment of metagenomic fragments against the genus pan-genome and compared to the recruitment patterns found in each analyzed species, which were obtained through the alignment of whole genomes to the genus pan-genome using the identity threshold of each species.

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# Testing the tomato root's microbiome assembly rules

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The soil is a heterogeneous environment due to its different solid fractions; representing micro habitats and selection sites for the microbial populations. Because of its complex nature, soil type and some of its physicochemical properties, such as pH and nutrient content have been proposed as the main factors driving the community structure within the rhizosphere, as opposed to other variables like the host's age and genotype. The rhizosphere is defined as the soil layer firmly attached to the roots of a plant, and it is the place where the mayor interactions occur between roots, pathogenic and beneficial soil bacteria, which can contribute to the growth and health of the plant. The rizosphere niche is a highly dynamic ecosystem which can be analyzed as different compartments, where the microbial diversity has been found to decrease when compared to the surrounding soil, suggesting that plants are actively recruiting a subset of the bacterial taxa present in the soil by means of the root exudates released into it, and which also serve as a nutrient source for microbes. In order to determine the variables influencing the bacterial community structure of the rhizosphere and endophytic compartment, tomato plants (*Solanum lycopersicum*) were grown in eight different soil types collected across the central region of Mexico and the root microbiome was characterized by 16S rRNA gene amplicon libraries. Physicochemical analysis were performed to gain insights about the soils' nutrient content and texture. Total Carbon, Nitrogen and Phosphorus were measured in the soils before and after planting. Physicochemical data showed variations in nutrient content in each time series and the differentiation of the soil types according to the measured parameters by means of a principal component analysis. The soils were dominated by Proteobacteria, Firmicutes, Verrucomicrobia, and Bacteroidetes phyla. The rhizosphere and endophytic compartments had a lower diversity (Shannon's H') than the surrounding soil, and showed an enrichment of the Bacteroidetes and Verrucomicrobia phyla, which could possibly be due to selective microbial recruitment by the plant roots. Finally, biomass production of each plant was determined as their dry weight and correlated with each nutrient. The plants with larger biomass were the ones grown in kastanozems, regosols, and planosol soils; in particular the kastanozem soil plants had twice the biomass than any other soil, we are examining the microbiome role, and configuration in the biomass production.

# Species interactions mediated by small RNAs

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Small RNAs (sRNAs) are 20-30 nucleotide long ribonucleotide molecules generally associated with gene repression activities, and a phenomenon known as RNA interference (RNAi). They are key regulators in many cellular processes in animals, plants and fungi. Some sRNAs move between cells of the same organism with the potential to cause a systemic RNAi response. This phenomenon has been described in plants and the nematode *Caenorhabditis elegans*. Recent reports show that sRNAs are able to move between organisms of different species, potentially causing an inter-species RNAi response. The clearest example of inter-species RNAi involves the fungus *Botrytis cinerea* and its host plants. This fungus encodes small RNAs that can reach *Arabidopsis thaliana* cytoplasm, hijack host RNAi proteins and down-regulate map kinase proteins involved in plant defense response [1]. Other cases of inter-species RNAi include human microRNAs that target *Plasmodium falciparum* in sickle cell anemia*;* vesicles containing microRNAs secreted by *Heligmosomoides polygyrus* that are internalized by mouse cells; and transgenic plants expressing exogenous RNAi precursors that achieve silencing of genes in pathogens such as fungi, oomycetes, nematodes and insects [2]. Inter-species RNAi is an emerging topic in symbiosis and RNA biology, however we lack a general perspective of its occurrence. Interestingly, all inter-species RNAi reports so far are of parasitic nature. This raises the question whether inter-species RNAi also occurs in mutualistic relationships. Nowadays, RNA high-throughput sequencing technologies allow quantitative small RNA profiling (sRNA-Seq) at a single nucleotide resolution. We aim to provide insights into the frequency of inter-species occurrence by a meta-analysis of transcriptome data of multiple host and interacting symbionts. We are developing a bioinformatic pipeline to disentangle dual RNA-Seq data of sets of symbionts. With our pipeline we are able to track the organism of origin of sRNAs, their generating loci and its nature (microRNAs, introns, exons, tRNAs, rRNAs, etc.), perform differential expression analyses of sRNA-producing loci, predict symbiosis relevant sRNAs and their possible targets. The fungus *Rhizopus microsporus* and its bacterial endosymbiont *Paraburkholderia rhizoxinica*, the entomopathogenic nematode *Steinernema carpocapsae* and its associated bacteria *Xenorhabdus nematophila*, the fungus *Trichoderma atroviridae* and *Arabidopsis thaliana* are some of the chosen symbiosis sets to begin our analysis. We used *B. cinerea* and *A. thaliana* sRNA-Seq data and genomes as a positive control while developing our pipeline. We aim to easily incorporate new symbiont genomes and new sRNA-Seq data to our meta-analysis, allowing a broader analysis that will lead to new insights into the phenomenon of inter-species RNAi.

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# *De novo* transcriptome sequencing by RNA-seq of an Asteraceae species to identify genes involved in the biosynthesis of secondary metabolites

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## ABSTRACT

Plants, especially the Asteraceae family, produce a large range of secondary metabolites with wide-ranging physical and chemical properties. These compounds have long been exploited through their use as flavors, pigments, and pharmaceuticals. However, most of these compounds occur in non-model plants for which genomic sequence information is not yet available. These secondary metabolites are tissue specific, such as root, flower, fruit, seed, or leaves. And also, are species specific, as occur in the Asteraceae family where acetylenic compounds are present mainly in the tribe Anthemideae and Heliantheae. This limited distribution may represent important chemotaxonomic criteria differentiating. Such characteristics make Asteraceae species a suitable model for studying the genes involved in the biosynthesis of secondary metabolites, specifically those are differentially expressed between tissues. Transcriptomic data mining is an efficient way to discover genes or gene families encoding enzymes involved in various metabolic pathways. Recently high-throughput sequencing of cDNA (RNA-seq) has emerged as powerful tools for transcriptome analysis. This technology has been applied to several non-model plants that produced important natural compounds. The objective of this work was sequencing by RNA-seq the transcriptome of one Asteraceae species to identify genes involved in the biosynthesis of secondary metabolites. Our species of interest was *Heliopsis longipes*. The selected tissues were roots and leaves. In order to sequence the transcriptome, we employed the platform Illumina/Solexa HiSeq 2500. RNA sequencing reads were assembled and clustered into 165,770 unigenes with an average length of 695 bases. NCBI nonredundant protein databases (NR) and Swiss-Prot database search anchored 81,682 unigenes (49 %) with functional annotations based on sequence similarities. Further unigenes were assigned with Gene Ontology (GO) in 10,413 terms distributed in the three major categories of GO: 24% for biological processes, 18% for molecular function, and 7% for cellular components. It was possible identified and characterized 56 novels genes involved in the fatty acid biosynthesis pathway in *H. longipes* for the first time. Comparative analysis of the transcriptomes by differential expression analysis identified 659 unigenes that were highly expressed in roots. Of this genes KEGG identified secondary metabolite pathways, covering the phenylpropanoid and terpenoids pathways, as well as carotenoid biosynthesis, amino acid metabolism, auxin-responsive, metabolism of nitrogen and lipid metabolism. Thus we have generated a large unigenes dataset in the transcriptome of *H. longipes* for further investigation.

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# "Cell death pathways *in silico* model based on biochemical tuple spaces for self-organizing coordination"

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## Introduction

The diverse pathways that cell death manifests itself, so as the interactions that occur between their molecular components, can be simulated through computational models for their comprehensive study. In this work, the bioinformatical platform *BTSSOC-Cellulat* was employed, since it offers robust computational approaches, capable of satisfying the crucial characteristics that govern the activity, interaction and evolution of complex biological signaling systems – situationality, adaptation, diversity, relationship consumer/provider, topology and location [Cárdenas-García et al., 2013].
The controlled modalities of cell death can be functionally classified into: (1) Apoptosis (intrinsic and extrinsic), (2) Anoikis, (3) Autophagy, (4) Cornification, (5) Entosis, (6) Mitotic Catastrophe, (7) Necroptosis, (8) Netosis, (9) Parthanatos and (10) Pyroptosis [Galuzzi et al., 2012]. Some of these are present in pathological conditions – Parkinson's disease, icthyosis, cerebrovascular accidents, and particularly cancer, among others, in agreement to what has been proposed by recent studies [Pietrocola et al., 2014; Miller et al., 2014; Ravindran et al., 2009; Fatokun et al., 2013; Cárdenas-García et al., 2013; Valdés et al., 2013].

## Materials and methods

Through revision of scientific literature, it was possible to obtain the pertinent information concerning the molecular components of cell death mechanisms, which contemplates the identification of the biochemical reactions, the cellular compartments in which they take place, the situation of temporality, the enzymatic values for each reaction, as well as the potential pathways that might cause the distinct death modalities to intersect.
As for biochemical reaction, they were modelled according to the form that the platform can accept for its correct performance: *aA+ bB -> cC* or the standard enzymatic equation: *E + S <-> ES -> E + P*.

## Results

The goal of the *in silico* experiments was the simulation of each of the pro-death and pro-survival signaling pathways separately in order to identify protein involved in more than one signaling pathway, which have not been reported in the literature. Due to the flexibility of the BTSSOC-Cellulat platform we were able to perform a number of simulations representing the networks that give pace to the initiation of the different cell death mechanisms, as well as the ones which annihilate them, allowing the cell to survive, so enabling it to become malignant in specific cases.

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# Identification of Transcription Factor Binding Sites AraC/XylS-Type in *Escherichia coli* K-12 using a new Phylogenetic Footprinting pipeline.

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The *in silico* identification of Transcription Factor Binding Sites (TFBSs) is a key issue for many Molecular Biology studies aimed to characterize the regulatory elements in genomic sequences. The goal of many algorithms developed to find TFBSs has been the identification of discrete sequence motifs that are overrepresented in a statistically significant manner in a given set of sequences where a TF is expected to bind. Despite their extensive use, the accuracies reached with these methods are still low. In many cases, the true TFBSs are excluded from the identification process or imprecisely identified, especially in those cases when the TFBSs correspond to low affinity sites.

In this sense, we have developed a new search tool, named PProCoM (Phylogenetic Profile of Consensus Motifs) which is based on the construction of Profiles obtained from a set of Consensus motifs of canonical Phylogenetic Footprinting studies using analysis windows of different sizes. In addition, PProCoM considers different important key issues such as the regulatory activation/repression nature the of the TFs, the preferential positions of the TFs in respect with the promoters or other TFBSs, the simple and complex regulation and the possibility of some TFs to bind cooperatively to adjacent low affinity sites.

Using PProCoM we have identified conserved directed repeat motifs that represent the TFBSs consensus of four TFs (AraC, FeaR, NimR and YqhC) of the AraC/XylS family in Gammaproteobacterias. Furthermore, a detailed analysis of these motifs allowed us to infer the possible activation role of the TFBSs in their corresponding intergenic sequences and to propose consistent molecular models that consider the presence/absence of the inducer of each one of the regulatory systems analyzed.

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# Análisis de los efectos epistáticos entre mutaciones que alteran la esperanza de vida en *Saccharomyces cerevisiae*

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Aging is a process which is characterized by the progressive accumulation of damage that leads to function loss in cells and tissues of an organism over time. It is a complex phenotype that depends on both genetic and environmental factors. With the aid of model organisms such as *Saccharomyces cerevisiae*, *Caenorhabditis elegans* and *Drosophila melanogaster*, genes and pathways affecting life expectancy have been identified, many of which are conserved across species. Since mutations that shorten or increase lifespan of an organism have only been studied independently, we know little about how the different cellular processes determine the rate of aging. Therefore we present an analysis of genetic interactions among mutations that alter lifespan in *S.cerevisiae* in hopes of understanding better the processes that regulate aging and determine if the genes which affect aging act on common or independent pathways.

We defined a group of 154 genes that had been previously reported to affect lifespan, have an homolog on humans and represent different functional classes. We constructed a collection of about 12,000 double and single knock-out mutants of the genes selected and competed the mutants against a wild type yeast to assess the relative lifespan of each mutant.

We show the frequencies of the relative lifespan of double knock-out mutants and contrast it to the distribution of relative lifespan of single knock-out mutants. From the comparison of single vs. double mutant lifespans we obtained a measure of interaction between a pair of genes that was used for constructing a genetic interaction network for the aging phenotype. We describe the frequency of positive, null or negative epistasis between genes of interest which prompts us to suggest which genes might act on common pathways or serve as antagonic or suppressor mutations. We also report the distribution of epistatic interactions among knock-out mutations that shorten or increase lifespan, showing how frequent is to have a second mutation that compensates or aggravates the effect of the first mutation. Additionally, we describe the main properties of the network of genetic interactions, such as the identity and density of hubs in the network, robustness, and similarity to other interaction networks like protein-protein interactions, transcriptional coexpression and genetic interaction network for other phenotypes.

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# Structural analysis of MrpA subunit from Na+/H+ antiporter complex of the archaeon *Methanosarcina acetivorans*

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*Methanosarcina acetivorans* is a methane-producing archaeon, capable to survive in oil wells, abyssal hydrothermal vents, and non-oxygenated sediments. Cation/proton antiporters are of fundamental importance and widely distributed in organisms from phylogenetic domains *Bacteria*, *Archaea* and *Eukarya*. The cation/proton antiporter-3 family, also called Mrp complexes, has been implicated in functions which include $Na^+/H^+$ antiport, $K^+/H^+$ antiport, pH homeostasis, resistance to bile salts, arsenite oxidation, pathogenesis and energy conversion. Mrp antiporters (MrpA, MrpD and MrpC) are homologous to the antiporter-like subunits (NuoL, NuoM/N, and NuoJ) of respiratory Complex I from the domains *Bacteria* and *Eukarya,* respectively. Understanding the antiport mechanism of Mrp complexes has been hindered by the lack of a crystal structure either from MrpA/D subunits or whole complex. In this work a complete molecular model of MrpA from *M. acetivorans* was constructed using NuoL/NuoJ subunits from respiratory Complex I from *E. coli* (PDB: 3RKO). The model shows the N-terminal domain of MrpA sharing a central core with NuoL composed by 14 TMS and a C-terminal extension called the "piston" composed of a predicted TMS and a long helix terminating with a TMS. However, the piston sequence is 50 residues less than the NuoL piston bringing the N-terminal and C-terminal domains of MrpA 30 Å closer. The C-terminal domain of MrpA is analogous to NuoJ of Complex. Proton translocation involves two half-closed symmetry-related channels that are linked forming a continuous path from the cytoplasm to the periplasm. TM7 and TM12 from MrpA are interrupted in the middle of the bilayer by an extended loop by P232 and P394 respectively (conserved in NuoL and NuoM), both helices probably participate in proton or ion transport by introducing flexibility as has been proposed in NuoL mechanism. The two channels are interconnected through TM8. Key residues in NuoL channel 1 (K229 and E144) and channel 2 (K399) are conserved in MrpA corresponding to K227, E141 and K402 that form a putative ion translocation route. Invariant residue H254 that links the two half channels in NuoL is conserved in H252 of MrpA. Like in NuoL, MrpA funnel is formed by two cavities connected by charged and polar conserved-residues (H252, K347, H343, T310 and S253), the cytoplasmic cavity is formed by R112, E141, S147, T171, K227 and T255; the perplasmic cavity contains S309, S312, Y316, H339, S401, K402, E403, T439, Y442 , and S443. Piston-like structure can drive conformational changes upon TM7 in the first channel, and bH motif could promote TM12 movement in the second channel. Indeed, the electrostatic potential determined for the MrpA_N domain from *M. acetivorans* showed that the cytoplasmic surface have a negatively charged patch formed by E360, E375, E454, D458, D460, this patch probably form the funnel face. In the periplasmic surface, residues E327, E403, E407, E411, E414, D510 highlight a strongly negatively charged patch.

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# Deciphering potential determinants for exosomal miRNAs package

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MicroRNAs (miRNAs) are endogenous ~22 nt long transcripts in metazoans that regulate the expression of most protein-coding messages by targeting mRNAs for cleavage or translational repression and thereby affect many developmental cell processes and cell fate decisions (bartel et al). In the last years it has been reported that extracellular microRNAs can participate in cell-to-cell communication through their packaging into exosomes. Exosomes are ~50 - 100 nm diameter vesicles of endosomal origin that can be released into the microenvironment by many different eukaryotic cells, thereby serving as vehicles for transferring cytosolic proteins, lipids, and RNA (valadi et al, 2007; raposo et al, 2013). Exosomes are formed as intraluminal vesicles by inward budding of the limiting membrane into the lumen of late endosomes (Meckes et. al., 2010) and can selectively package miRNAs during their formation. Indeed, It has been reported that exosomal miRNAs play an active role during immunological synapse formation (Mittelbrunn et al. 2011), pathogenic immuno-repression (Buck et al. 2014; Pegtel et al. 2011) and cancer (Zhou et al. 2014) by reprogramming gene expression of target cells. Exosomal miRNAs have great potential uses in therapeutics or as biomarkers for disease. However, the factors that determine the packaging of miRNAs within exosomes are still not well understood. There are, however, large amounts of small RNA sequencing data available in open access databases, which can be re-analyzed to answer basic questions and test hypotheses regarding the packaging of miRNAs into exosomes. The motivation of this project is to identify and compare potential factors that determine the specificity of miRNA packaging in different model organisms through the computational analysis of raw small RNA-seq data.

**key words:** microRNAs, ncRNAs, exosomes, extracellular vesicles, cell communication, cancer, microbial infection, bioinformatics

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# Arbuscular mycorrhiza symbiosis induces a major transcriptional reprogramming of the common bean nutrient transporter families

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## ABSTRACT

Macro and micro nutrient are important components in synthesis of key cellular bio-molecules and plants cannot grow without reliable supply of these nutrients. Host plant uptake the nutrients directly through roots, on the other hand Arbuscular mycorrhizal (AM) symbiosis improves host plant nutrient uptake under low nutrient availability. The role of AM symbiosis on phosphate has been extensively studied and other reports show significant increase of N, S and Fe uptake in the mycorrhized roots. Host plant transporter proteins are predicted to have an important role in the nutrients uptake of AM symbiosis however; the current knowledge of AM fungi associated transporters is relatively patchy. To fill these gaps we performed transcriptome profiling of common bean (*Phaseolus vulgaris*) mycorrhized and non-mycorrhized roots by RNA-seq. Herein, we present comprehensive transcriptome profiling of more than 15 types of transporter families and their expression pattern during AM fungi colonization. Further, we identify several putative candidate genes that are induced in presence of AM fungi. Promoter analysis of some of the key transporter genes are under progress to reveal the AM fungi associated expression in the common bean. This work is supported by PAPIIT (DGAPA-UNAM) grant no. IA205115 to MK.A.

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# Designing of a bioinformatic system for the 16S ribosomal sequences massive analysis

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16S rRNA is the genomic marker commonly used in metagenomics studies in recent years to establish phylogenetic relationships among prokaryotes. This type of analysis has had a huge impact on bacterial taxonomy. The sequence of the 16S gene is used as a molecular marker due to a number of features: a) 16S gene is presented in all bacteria, which makes it a target for molecular identification; b) its structure and function remained constant in the evolutionary process, so the alterations in the sequence probably reflect random changes (1-2% variation in the sequence every 50 million years) that contain enough variability to distinguish not only the farthest, but also the nearest organisms (16S gene contains nine variables V1-V9 sites and ten preserved sites); c) because it is relatively easy to sequence, there are many databases and are constantly growing[1].

For amplification of the 16S rRNA it is used in oligonucleotides designed, conserved regions near the 5 'and 3' of gene end to amplify a fragment of 1500 bp. However, it has been shown that the identification does not require the complete sequence, it can be used a smaller region (about 600 bp). This region in the position 804-1392 bp of the 16S gene includes five variable sites (part of the V4, V5-V8) and four preserved, being an ideal region to identify and classify (at genus level) bacterial species because it presents variability and degree of conservation in its structure[2].

Currently, there are several tools for the identification and bacterial classification; such as the NCBI BLAST and EzTaxon as identification tools and Clustal Omega EMBL as classification tool. To analyze information with different bioinformatics tools as above, it is required by the user prior knowledge in the area of computation or in some situations, knowledge in the area of programming.

The present study aims to develop and implement a bioinformatic system for ribosomal sequences massive analysis in the region from 804 to 1392 bp of the 16S gene that will make the process of identification and classification of bacterial species in a single step, and has a graphical interface to help users in exploring the data and the interpretation of results. The unified database will be used as a reference against massive sequencing files that users of the system will upload.

To achieve this, it is needed to download and combine information from three databases (RDP, Greengenes and Silva), filtering unique sequences in the data set, obtaining a unified database. Through the alignment of a forward primer with two degenerations (AGATTAGATACCCDRGTAGT-six sets of primers) and a reverse primer with one degeneration (ACGGGCGGTGTGTRC-two sets of primers) against the unified database, it will be obtained the region of interest (804-1392 bp). The whole cut sequences, will be grouped by classification algorithms. For the logical architecture of the system it will have three layers (data, business logic and presentation), the design of the data layer will be done with Manager Database System (DBMS) MySQL from data sets created with classification algorithms. The business logic layer will search algorithms to identify sequences entered by the user in the pooled database and the presentation layer will contain intuitive and easy to use interfaces for the user.

The three databases belong to the smaller ribosomal subunit (SSU 126) and were downloaded from the official website of Silva (https://www.arb-silva.de/). 7976718 sequences of the three sources of information were obtained, of which 1710558 are unique sequences. 483,800 sequences belong to the region of interest (804-1392 bp); 640,823 sequences did not meet the length of the region. 119859 sequences did not recognize the forward primer and the remaining 466,076 sequences did not recognize the reverse primer.

It is proposed to study the possibility of further degeneration in cut primers. In addition, the bacterial diversity of cut sequences will be met by calculating the Operational Taxonomic Units (OTUs).

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# Effect of stimulation with interleukin-10 on peripheral blood mononuclear cells: a bioinformatic analysis

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Interleukin 10 (IL-10) is an anti-inflammatory cytokine. IL-10 can inhibit production of pro-inflammatory cytokines, antigen presentation, and cell proliferation. On other hand, IL-10 can co-stimulate B-cell activation. It can also co-stimulate natural killer (NK) cell proliferation and cytokine production. Moreover, changes in this molecule have been associated with diseases, such as rheumatoid arthritis, psoriasis, systemic lupus erythematosus, diabetes, obesity, among other. Therefore, it is very important to know more about the effects of IL-10, especially the genes regulated by this molecule and metabolic pathways in which they participate.

The gene expression profile GSE43700 was obtained from the public functional genomics data repository gene expression omnibus (GEO) database. These data are derived from mononuclear cells of clinically healthy subjects (n = 8), half of them stimulated by IL-10 (10ng/ml). These data were analyzed with the software GEO2R, to identify genes that are differentially expressed (DEGs). The genes obtained from the analysis were introduced to WEBgestalt database in order to identify metabolic pathways in which the genes involved. Then the database STRING to infer the interactions between these genes was used.

A total of 1,598 DEGs were found in mononuclear cells, 842 of these were upregulated and 756 downregulated. Analysis with database Webgestalt revealed that upregulated genes are involved in metabolic pathways related to the immune system, as it was expected; but also metabolic pathways related to infectious diseases and cancer were found. Interestingly, the signaling pathway of insulin also was associated with DEGs by the action of IL-10, which supports previous reports of the relationship between the immune system, obesity and diabetes. In relation to downregulated genes, only 4 metabolic pathways were found enriched, against 27 of the upregulated genes. These were: Hematopoietic cell lineage, Cytokine-cytokine receptor interaction, Osteoclast differentiation and Hepatitis C. Analysis with STRING showed great connectivity between genes. The results shown support previous reports, which display the IL-10 as a pleiotropic molecule, which acts through mononuclear cells participating in a variety of biological processes and diseases, not only those related to the immune system .

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# "Environmental gene-content homoplasy and evolution of *Bacillus*"

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The *Bacillus* genus comprises Gram-positives bacteria, aerobic or facultative anaerobic, rod-shaped, that have the ability to form endospores. Members of this genus are considered ubiquitous, since they had been isolated from a wide range of environments, such as terrestrial as aquatic. However, is not clear if their presence in such variety of environments, is due to their ability to colonize new environments, or due to unusual spore resistance and spore dispersal by air and water.

Some authors have suggested the existence of an aquatic *Bacillus'* group within the genus. Despite the numerous *Bacillus* genome sequences available, isolated from different environments, few works have analyzed the relationship between the evolutionary history of *Bacillus* spp. and the environment where they are found. In this work, we analyzed the relationship between the evolutionary history of *Bacillus* spp. and the environment from which they have been isolated, focusing our attention mainly on the aquatic *Bacillus* spp.. Furthermore, we analyzed if there was a relationship between the environment and the functional gene content.

The phylogenetic reconstruction, using the core genome, suggested that the aquatic *Bacillus* spp. do not form a monophyletic group. In contrast, a clustering analysis based on gene content groups together the aquatic *Bacillus* spp. that appear separated in the phylogenetic tree. These results suggest that *Bacillus* spp. isolated from similar environments share more functions between them than would be expected from their polyphyletic origins. The functional gene content analysis also suggests the presence of functions specific for each environment.

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# *De Novo* transcriptome assembly of telomerase-negative strains of *Ustilago maydis*

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*Ustilago maydis*, the basidiomycetous fungus causative of the corn smut disease exhibits two styles of life: haploid saprophytic sporidia, and dikaryotic pathogenic hyphae. The switching in the life style is concomitant to the change in morphology and ploidy and many genes are exclusive from each phase of life. We chose the valuable model system fungus *U. maydis* for research in molecular mechanisms involved in the synthesis, maintenance and healing of telomeres. Telomeres are nucleoproteic complexes located in the very end of the chromosomes. They are composed with DNA, specific telomere-binding proteins and RNA; its function is preventing the recognition of chromosome termini as broken DNA by the machinery involved in double strand break (DBS) repair, to avoid DNA degradation and occurrence of aberrant recombination events at chromosome ends that could fuse them, and to serve as substrate for its own replication and lengthening. Telomere is essential for eukaryotic cells, as it is the enzyme engaged in the synthesis of telomeres named telomerase. Limiting components of the enzyme are the catalytic subunit known as telomerase reverse transcriptase (TERT) and the RNA moiety (known as TR), which port the template for telomere synthesis. Null mutants in TERT or TR genes senesce, their life span shorten, and ultimately die, however, telomerase-negative mutants eventually give rise to survivors, which maintain telomere function by mechanisms that involve up- or down-regulation of several as 30 genes or more that enable two main pathways of recombination based enlargement of telomere sequences, or amplification of telomere associated sequences. As nuclear ncRNA metabolism is underlying the telomere RNA metabolism, in this work we initiate our studies of global transcriptional-expression analysis of telomerase negative mutants *trt1-1* and *trt1-2* and of their parental WT strain 521 of *U. maydis.* We extracted high-quality total RNA from the three strains and it was kindly processed and sequenced in LANGEBIO-CINVESTAV Campus Irapuato. Transcriptome was obtained by RNA-seq of poly (A) transcripts with the Ion-Torrent Next-Generation platform from Life Sciences by duplicate. Assemble was made using the free software Trinity. Preliminary identification of 172,290 transcripts was made using the Trinity-RNA-Seq form assemble of reads obtained from transcriptomes of WT and A mutant of *trt1* in duplicate experiments; length of transcripts was from 201 bp to 5049 with a total of assembled bases of 67,167,470 bp and N50 = 395 bp, Similar analysis was made from WT and B mutant, in which replicates were used again to find 194,904 transcripts were identified from 72,896,064 bp assembled. Length of transcripts form 201 pb to 5004, N50 = 374.
Differences in transcripts abundance are showed as preliminary result.

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# Phylogenetic analysis of the chromate ion transporter (CHR) superfamily revisited.

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The ChrA protein confers resistance to the toxic ion chromate through the chromate efflux from the cytoplasm. ChrA proteins belong to the chromate ion transporter (CHR) superfamily, composed of two families: large bidomain proteins (LCHR) and short monodomain proteins (SCHR). LCHR proteins are composed of two homologous domains with an oppositely-oriented membrane topology; *schr* genes are organized, with very few exceptions, as tandem pairs of genes whose resultant proteins also possess oppositely-oriented membrane topology. Thus, all CHRs characterized are functional heterodimers. Diaz-Magaña et al. (J. Bacteriol 2009, 191:5441) showed that expression of one *schr* gene alone did not confer chromate resistance, and therefore the tandem pair of genes are required for function. Interestingly, some bacterial *schr* genes are organized as a single-copy gene, and therefore, its role as chromate ion transporter is dubious. The aim of this work was to perform phylogenetic and topological analyses of single *schr* genes, and to identify the proteins associated to these genes. Results obtained showed that single *schr* genes comprise a non-monophyletic group with a non-dual topological orientation. Furthermore, the genes found in the operons that contain single *schr* genes are not related to the genes found with the tandem pairs of *schr* genes. All this suggest that the function of single *schr* genes is not related to chromate ion resistance.

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# Comparative analysis of the protease sequence of *Totoaba macdonaldi*

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The most important proteases in the digestion process are trypsin, chymotrypsin and pepsin. The first two are endoproteases that belong to the serine protease family, while the last one belongs to the aspartic proteases group. Serine proteases are very important because of their wide applications in the food industry. Recent studies, based on the amino acid sequence have been shown that the trypsin isoforms I and III isolated from marine species are cold-adapted enzymes, which are characterized by high activity at low temperatures, a high catalytic efficiency and an overall flexible structure. *Totoaba macdonaldi* is endemic specie of the Gulf of California, whose natural population has been diminished considerably by illegal fishing, due to the marketing of its swim bladder. This is the only member of the *Scinidae* family, in which further studies are required to support its repopulation. In this work, we identified and analyzed important residues totoaba proteases, conserved regions or important residues of this family of proteins, and found that proteases from Totoaba fish presented high percentages of homology with other proteases already reported as cold-adapted. By performing molecular modeling, we identified characteristics regions of these proteins. Therefore according to their similarity sequence with other cDNA of cold-adapted enzymes, it suggests that it could be an enzyme with activity at low temperatures and studies could be realized for its further biotechnology application.

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# Genome assembly and comparative genomics analysis of Ca. *Mycoplasma haemobos* strain INIFAP01, the first hemotrophic mycoplasma identified in Mexico.

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Hemotrophic mycoplasmas (hemoplasmas) are a group of wall-less bacteria and animal pathogens of the Mollicutes class. These bacteria are found in a wide range of host species, including mammals. Currently, the genomes of ten hemoplasmas have been completely sequenced and deposited in the GenBank database: *Mycoplasma haemocanis* Illinois, *M. haemofelis* Langford 1, *M. haemofelis* Ohio2, *M. suis* Illinois, *M. suis* KI3806, *M. parvum* Indiana, Candidatus *M. haemolamae* Purdue, Ca. *M. haemominutum* Birmingham 1, *M. wenyonii* Massachusetts and *M. ovis* Michigan. Hemoplasmas have small genomes sizes varying from 513 Kb to 1.1 Mb. These organisms were subjected to a genome reduction resulting in a concise metabolism, capable of surviving often evading the immune responses and establishing chronic infection. Recently, CeNID-PaVet, INIFAP reported a bacterial strain of the genus *Mycoplasma*, an uncultivated hemoplasma isolated from blood of sick cattle from Chihuahua, Mexico. Bovine hemoplasmas can be found attached to erythrocytes surface of cattle and may act in a synergistic manner when coinfections are present. The aim of this work is to reveal and compare genomic features of Ca. *Mycoplasma* sp. INIFAP01 and the relationship with other hemoplasma species. Furthermore, identification of genes involved in pathogenicity, coinfections and synergistic mechanisms associated with the cattle disease has a relevant interest.

In this study, we report the draft genome of Ca. *Mycoplasma* sp. INIFAP01 which consists of 935,638 bp total length. In total, 18 contigs were produced using *de novo* assembly with SPAdes program with $N_{50}$ contig size of 256,799 bp, G+C content of 30.46% and ~48X coverage. Phylogenetic analyses with 16S rRNA gene showed that *Ca. Mycoplasma* sp. INIFAP01 clusters with *Mycoplasma haemobos* species, these hemoplasmas are divided into two main groups: group “A” containing *M. haemobos*, *M. haemocanis* and *M. haemofelis* species; and group “B” containing *M. suis*, *M. parvum*, *M. haemolamae*, *M. haemominutum*, *M. wenyonii* and *M. ovis* species. This result was confirmed by structural analysis of the 16S - Internal Transcribed Spacer (ITS) - 23S region sequence. All 18 contigs were analyzed with RAST server, identifying 1,216 open reading frames (ORFs), 1,184 coding sequences (CDS) and 850 hypothetical proteins. The CDS were used to identify clusters of orthologous groups (COGs) resulting 277 CDS with predicted function. COGs classification showed that defense mechanisms have the largest number of CDS with 33 results.

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# V2 promoter variant in silico analysis of human gene *ST3Gal4*

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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, B1, B2, B3 and BX, which are produced by alternative splicing and the usage of different promoters. Isoforms type B 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. Bx transcript, which we will referred to as V2 variant, has been detected in A549 cells where it`s interaction with TNF induces an over-expression of both sLex and 6-sulfo-sLex antigens. 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 of such gene.

Material and Methods: Vega Genome Browser was used to download the ST3GAL4 cDNA full sequence including introns and exons. The obtained sequence was then examined on NCBI to determinate the number variants and their localization. For promoter V2, we decided to analyzed 1,500bp upstream from mRNA_V2 variant. Eukaryotic Promoter Database, Transcriptional Regulatory Element Database and Softberry Software, were used to identify promoter region, putative transcription factor binding sites, and transcription start site respectively.

Results: After NCBI analysis, we found that variants V1, V2, and V4 do encode isoforms B1, Bx, and B3, previously reported by Kitagawa, but the V3 variant does not match Kitagawa B2 isoform as it is located 2,000 bp upstream to the site that he describes. in silico analysis of the V2 variant found different putative transcription factor binding sites for SP1, AP-1, TNFα, RARα, Lun-1 and two sites for TBP and TFIID, additionally an INR box site was located.

Conclusions: Our results give a clue to discover how the transcription process takes place for V2 variant in ST3GAL4 gene. However, experimental assays will be necessary to further understand the transcriptional regulation of glycotransferase ST3GAL4 and analyze it's behavior both in healthy conditions and disease development.

# Constraint-based modeling in cervical cancer, a multiomics approach

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Cervical Uterine Cancer is one of the most important cancers worldwide and a central issue to solve in our country. The major etiological factor is the human papillomavirus (HPV) which include a variety of subtypes such as the HPV16 and HPV18. Among the modifications that the HPV genes cause are the alterations transcriptional regulatory mechanism and metabolism, this latter one of the new hallmarks of cancer. Understanding the metabolic alterations that support the normal and cancer phenotype in human samples is a primer challenge to overcome for uncovering the principles by which tumorigenesis evolve and eventually how to delay its progression.

With the purpose to elucidate these metabolic alterations among normal and cancer samples, this project focuses in the computational analysis of high-throughput “multiomics” data at three different levels: transcriptome, proteome and metabolome. These data were obtained from public databases including different technologies of mRNA microarrays coming from Gene Expression Omnibus database and proteome data from the Human Protein Atlas database. Furthermore, metabolome data were obtained in the laboratory from HeLa and HaCaT cell lines. Together, these data contributed to create different metabolic tissue specific models that represents normal biopsies (non-neoplastic), cervical cancer biopsies, HaCaT cell line as a normal cell line and HeLa as the cancer cell line.

Our analysis let us to identify some metabolic alterations among these samples, some of them reported as fundamental to propelled the typical phenotype for cervical cancer cells. Finally, each one of these models represent a computational platform with capacities to understand the different metabolic capabilities of the cervix epithelial cells and suggest hypotheses of what extend the HPV could contribute to modify the metabolism to induce the cancer phenotype.

# Extracellular small RNAs during parasite-host communication

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The discovery of stable small RNAs moving between organisms, suggests the existence of RNA-based communication, with implications for ecology, agriculture and disease. We have previously reported the presence of exosomes with small RNAs and a worm-specific Argonaute in the secretory products of the parasitic nematode *Heligmosomoides polygyrus* [1]. We are now further characterizing the molecular content of these exosomes, and particularly want to understand the function of the different types of small RNAs during infection.

We are using biochemical and computational methods to characterize exosome and supernatant samples from *H. polygyrus* excretory and secretory (HES) products, including small RNA-seq of mono and poly-phosphate libraries. To help us characterize these results, we used long single-molecule sequencing (PacBio) to improve the *H. polygyrus* genome assembly and annotation. We have now determined HES-ncRNA producing regions according to how reads cluster on the genome.

Certain classes of these HES-ncRNAs are enriched in exosomes, compared to the supernatant samples. These include short and full-length yRNAs, 22G siRNAs, microRNAs, and specific fragments from tRNAs and rRNAs. The polyphosphate libraries are particularly enriched in 22G siRNAs, consistent with their expected biogenesis, suggesting that a complex RNA-interference machinery is involved in producing the exosome content.

These results will help us understand the functions of exosomes and their cargo during *H. polygyrus* parasitism, and highlight a versatile role for RNA during interactions between organisms.

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# Molecular dynamics studies of CssIV scorpion β-toxin binding to the voltage gated $Na_v1.2$

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## Abstract

Voltage-gated sodium (Nav) channels are transmembrane protein complexes that act as receptors for the scorpion β-toxins. Scorpion β-toxins modify the activity of Nav channels, thereby producing neurotoxic effects in diverse organisms, particularly in humans. The β-toxin CssIV is a neurotoxin isolated from the *Centuroides suffusus suffusus* mexican scorpion. The experimentally reported CssIV binding site of the receptor involves the extracellular loops S1-S2 and S3-S4 of domain II, and in the SS2-S6 loop of domain III. Until the crystallographic structure becomes available, molecular docking methods and molecular dynamics simulations are excellent tools to study the binding modes of CssIV to the voltage-sensing domains of Nav1.2. We have performed all-atom 50 ns MD simulations of the CssIV/Nav1.2 complex embedded in a lipid bilayer. In this report we depict the main amino acid interactions pairs, as predicted by experiments, their distance fluctuation along the simulation time, and the root-mean-square deviation of the complex.

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# Analysis of the dynamic of deletions in mitochondrial DNA from human somatic cells

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The mitochondrion is a cellular organelle whose main function on the cell is the energy production through oxidative phosphorylation process. Mitochondria contain their own double-stranded circular DNA with fundamental encoding genes for the oxidative phosphorylation function; however, in the mitochondrial genome different types of recombination events occurs, being deletions the most common and their accumulation during the life of an individual could cause a wide of neurological and debilitating diseases, the mitochondrial diseases. In this work we present a method of isolating circular DNA from dental pulp and peripheral blood for the identification and quantification of patterns in mitochondrial DNA deletions from apparently healthy individuals through massive sequencing by Ilumina Hiseq and bioinformatic analysis. With the development of this method we can find a full range of deletions and other variants normally present in cells from healthy individuals. The analysis of the set of deletions in mitochondrial DNA will reveal the dynamics of deletions in the mitochondrial genome of healthy individuals and the development of a method that allows us to detect deletions of different size and even the detection of those with low frequency that may be the basis for a new method of diagnosis that allows the complete identification of recombinations with minimal technical bias.

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# Transcriptomic analysis of the halophile adaptation responses of *Aspergillus caesiellus* in a lignocellulosic solid-state fermentation.

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The molecular characterization of halophilic fungi with lignocellulolytic activity is attractive due to potential biotechnological use and the contribution to a better understanding of the molecular mechanisms responsible for fungal physiology in such conditions. *Aspergillus caesiellus* is among the few examples of halophile ascomycetes and has been shown to grow on diverse lignocellulosic materials. Therefore, we aimed to characterize the transcriptomic responses of this strain to salinity when growing in wheat straw as sole carbon source. For this purpose, we characterized the growth of *A. caesiellus* in a semi-solid fermentation of wheat straw without NaCl and in the presence of 0.5M and 2.0M of this salt. 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. Our data show that while there is no significant down- or upregulation of the "lignocellulolitic genes", some of these are highly expressed under salinity conditions, suggesting the presence of extremophilic enzymes in this strain. Furthermore, the analysis of the halophilia mechanisms in this fungus showed that hydrophobins (a protein family that is present exclusively in fungi) have a very strong differential expression. This behavior has been previously described for a basidiomycete (*Wallemia ichthiophaga*), but the role of the "halophilic hydrophobins" has not been studied. This would represent a novel mechanism of halophilia in fungi when compared to other halophilic microorganisms.

# Functional conservation of neuronal microRNAs

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MicroRNAs (miRNAs) are small non-coding RNAs of ~22 nucleotides in length. They are involved in post-transcriptional regulation of most protein-coding transcripts by deadenylation and translational repression [1]. In animals, target recognition is achieved by binding between the seed region, 6 to 8 nucleotides at the 5' end of the miRNA, to the 3'UTR of a transcript. Due to this, animal miRNAs can potentially bind to thousands of target mRNAs. Expansions of miRNA families during bilaterian evolution correlate with gains in morphological complexity [2], and there are 19 conserved miRNA families that are expressed in neuronal tissues [3]. Nevertheless, even for highly conserved miRNAs, their predicted targets are not shared between distant lineages [4]. This could imply that the function of miRNAs is not highly conserved. Instead, we propose that the conserved function of a miRNA is to regulate the same cellular processes even though individual targets diverge. To explore this hypothesis we focused on the highly conserved neuronal miR-124, to see if the biological processes it affects are conserved. We first analyzed human and mouse gene expression profiles where the function of miR-124 is expected to be important. These included multiple tissue samples, and cell-lines over-expressing miR-124. We then performed seed enrichment analysis on the list of differentially expressed genes. Accordingly, we defined three subsets of genes: downregulated genes with functional miRNA targets, downregulated genes with no potential miRNA target, and seed depleted upregulated genes. Within these gene sets we searched for over-represented Gene Ontology terms. Between mouse and human the enriched GO terms that caught our interest are involved in stress response, among the downregulated set of genes, and nervous system functions, within the upregulated set of genes. We are now comparing with other bilaterians to see if these functions are conserved, and are performing similar analyses with other conserved neuronal miRNAs.

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# Transcriptional modification of Fma1, Fma2 y Pca1 genes in the model of chronological aging of *Schizosaccharomyces pombe*

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Aging research has experienced an unprecedented advance over recent years, particularly with the discovery that the rate of aging is controlled, by genetic pathways and biochemical processes conserved in evolution. The study of the molecular basis of aging has allowed to elucidate some of the process that represent common denominators of aging in different organisms, such as genomic instability, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, among others. During aging to maintain cellular homeostasis is necessary the regulation processes of cell cycle and apoptosis are finely coordinated, as well as the suitable protease activity for the correct processing of nascent proteins. Has been identified proteins as methionine aminopeptidase 1 and 2 (MAP1 and MAP2) are involved in cell cycle regulation, on the other hand, participation of Metacaspase 1 in the apoptosis process; In this study, through PCR Real Time screening, we analyzed the mRNA levels of FMA1, FMA2 y PCA1 genes in the yeast *Schizosaccharomyces pombe* with chronological aging. We used yeast with 2 days (young cells) and 6 y 8 days of culture (aged cells). In the analysis of proteins involved in protein processing and regulator of cell cycle, in this work it was observed that with 8 days of culture the level of FMA1 mRNA decreases while the FMA2 mRNA increases, so we can infer that the increase of FMA2 could be to compensate the decrease of FMA1, because it has been identified the activity of MAPs is essential for viability and growth cell, being more serious the effect of MAP1 deletion, because when the MAP1 or MAP2 activity are inhibited this leads to apoptosis, by the cell cycle deregulation. Regarding the monitoring of apoptosis, we observed PCA1 mRNA levels are decreased from 6 days in culture, so we can infer that the apoptosis process is dysregulated leading to a stress state in the cells population that eventually it leads to aging.

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# Genomic sequencing of *Herbaspirillum* sp. strain TQ07 and characterization of the metabolic pathway involved in chloranilic acid degradation

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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 elucidate in detail the metabolic capacity that certain microorganisms have for remove toxic compounds by biodegradation. In this work, the genome of *Herbaspirillum sp.* strain TQ07 was completely sequenced. This strain use 2,5-dichloro-3,6-dihydroxybenzo-1,4-quinone (chloranilic acid, CA) as only source of carbon and energy and 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 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 (of which 41 were longer than 1 kb), with 60.31 GC%. Genomic annotation was performed via RAST using the genus *Herbaspirillum* as reference, resulting in 4571 annotated features; two of these contigs contain putative genes related to CA degradation. The strain TQ07 spontaneously loses the ability to degrade CA when grown in rich media; this phenotype is attributed to a recombination mechanism that causes the loss of an 80 Kb genomic island. Also we found, that only *Burkholderia cenocepacia* strain DDS 22E -1 has a set of homologous genes similar in number, orientation and genomic distribution of 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. Additionally, the genes involved in the degradation of CA are not present in other strains of the genus *Herbaspirillum* which reinforces the hypothesis that they were acquired by TQ07 strain through a horizontal transfer. Bioinformatics analyses are in develop to identify another genes involved in the degradation of CA like dioxygenases. In fact, in one of the contigs related to CA degradation we found a catechol-2,3-dioxygenase pathway.

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# Pore-forming mechanism of the antimicrobial peptide Pandinin-2.

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Over the last decade several disease-causing microbes have become resistant to antibiotic drug therapy and came to be an important public health problem. Therefore there is an urgent need to develop new antimicrobial drugs in order to surmount the increasing resistance of pathogens to existing antibiotics. Naturally occurring antibiotics are a good place to start. A wide variety of organisms produce antimicrobial peptides (AMPs) as part of their defense system against pathogens. Most AMPs interact with lipid membranes causing cell lysis. The action of AMPs is a competition of the hydrophobic interactions between the side chains of the peptides and the hydrophobic region of lipid molecules, as well as the intra peptide interaction. In recent years AMPs have been considered as potential antibiotics since they act mainly against bacteria and fungi. Pandinin-2 is an amphipathic pore forming antimicrobial peptide isolated from the venom of the African scorpion *Pandinus imperator*. This peptide exhibits activity against bacteria as well as a strong hemolytic activity. Molecular dynamics (MD) simulations have been performed to analyze the structure, dynamics and pore-forming mechanism of Pandinin-2. Two membrane models, POPC and POPG, were used to understand how the phospholipid differences affect the conformation and mechanism of Pandinin-2 upon these membranes. The results indicate that the peptides are electrostatically attracted to the two types of membrane. When Pandinin-2 lies on the surface of the membrane layer it adopts a disordered structure which starts at the $COO^-$ and $NH3^+$ ends while the central residues retain their secondary structure. After 500ns of simulation, Pandinin-2 shows a toroidal pore in both membrane models.

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# BIK Interacts with coding and non-coding regions of the Breast Cancer MDA-MB-231 cells

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## Abstract

**BIK** (BCL2-Interacting Killer (Apoptosis-Inducing)) was initially characterized as a pro-apoptotic protein, but recent studies from our laboratory indicated its involvement in breast cancer **(BC)** malignancy. In general, **BIK** had been identified as a cytoplasmic protein; however, confocal microscopy revealed its presence into the nucleus of the **BC MDA-MB-231 (M231)** cells and the significance of this is unknown. Based on this, in the present study we demonstrated the physical interaction of **BIK** with the genome of the **M231** cells by **ChIP-chip** and its significance was bioinformatically analyzed. Results showed for the first time the association of **BIK** with both coding and non-coding regions of the human genome –specifically with that of **M231** cells-. Importantly, BIK was shown to be associated with the promoter regions of **eighty-three** genes and long-noncoding RNA **(lncRNA)** and microRNA **(miRNA)** ***loci***. Real-time PCR demonstrated that **BIK** interference, with siRNA, altered the expression of some of these eighty three genes, as well as that of miR-100-5p and -3p. Our results showed for the first time the interaction of **BIK** with coding and non-coding regions of the genome of the **M231** cells, which strongly suggest BIK involvement in gene regulation.

**Keywords:** BIK. MDA-MB-231 cells. ChIP-chip. Data mining. Protein interactome.

**Area:** Systems Biology and Bioinformatics

# Towards an understanding of the mechanism of a microtubule-length regulator: the kinesin-8 Kip3

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The kinesin-8 family of motors is best known for its regulatory role in controlling microtubule dynamics. Kinesin-8 motors play a conserved key role in microtubule length control and in the regulation of spindle size. Kinesin-8s are highly processive plus-end directed motors that are able to dwell at the plus-end of the microtubule and alter its dynamics. Kip3, the yeast kinesin-8, can actively depolymerize GMPCPP-stabilized microtubules and has been shown to be a catastrophe factor on dynamic microtubules in vitro. Although the importance of spindle length regulation by kinesin-8s in a variety of cell types and organisms is well established, the structural elements that uniquely allow for the dual-functions of Kip3 are unknown. Through domain swapping analysis and biophysical characterization of modular elements in the motor domain we identify the minimal elements required for microtubule end-recognition and destabilization. We demonstrate that motility and depolymerization are separable activities of Kip3. The yeast kinesin-8 interacts differently with straight and curved tubulin and this differential binding underlies its ability to walk and depolymerize microtubules at the plus-end.

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# Crystallization of copper chaperon LVATX from *Litopenaeus vannamei* (Penaeidae) in binding to different metals

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Many enzymes employ transition metal ions as specific cofactors [1]. In recent years, metallochaperones family was described as their function as escorts on intracellular trafficking of metal ions. Metallochaperones protect, guide and deliver the correct metal cofactor to their correct active site [2] that is the reason metallochaperones are important to biological processes such as respiration, photosynthesis, toxic agents defense and others [3].

One of the metallochaperones mechanisms include copper chaperones that bind intracelular copper and ensure proper trafficking to downstream targets via protein - protein interactions [4]. LVATX is an antioxidant copper chaperon (ATX1 like) from *L. vannamei (*white shrimp), the most important commercial shrimp specie in Latin America [5]. The crystallographic structures of ATX1 like chaperones have been determined in presence of Hg, Cd, Cu and other metals, indicating the possible union of other metal ions to LVATX metal binding site.

The gene of copper chaperon LVATX cloned in pJExpress404 (DNA 2.0) was expressed in *Escherichia coli* BL21 (DE3) strain and purified by affinity chromatography. Crystallization trials were made by the micro batch method and sitting drop system using a Mosquito LCP crystallization robot. Crystals were obtained by the two methods before mentioned, however they have not been tested yet under an X-ray beam.

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# Possible existence of microsomes in which takes place the degradation of polycyclic aromatic hydrocarbons

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Polycyclic aromatic hydrocarbons (PAH) are hydrophobic organic compounds which are formed of two or more benzene rings, are toxic and mutagenic; they are ubiquitous in the environment and as sub-products of human activities, many of them remain in the ecosystem for years becauseis low its water solubility and absorption (Peng *et. al.*, 2008; Seo*et. al.*, 2009).

It is knowing that peroxisomes are eukaryotic organelles with an important function in lipid metabolism and xenobiotic detoxification, included PAH degradation (Fukui *et. al.*, 1975; Tanaka *et. al.*, 1982; Veenhuis*et. al.*, 1989). However, in the YR-1 strain we have found evidence that suggests the existence of different microsomes to peroxisomes in which takes place PAH degradation.

The YR-1 strain of *Mucorcircinelloides* has been isolated from soil contaminated with oil in Salamanca, Guanajuato, and has been demonstrated the ability of this strain to use aliphatic and aromatics hydrocarbons as the sole source of carbon and energy (Silva and Zazueta 2005; Zazueta*et. al.*, 2009; Silva *et. al.*, 2009; Camacho *et. al.*, 2014).

The first steps for the degradation of PAHs include the participation of a monooxygenase (MO) and a dihydrodiol dehydrogenase (DHD), detecting these activities is possible to infer where it is carrying out the PAH degradation, at least in the initial stage.

Spores of *M. circinelloides* YR-1 were incubated aerobically in salts minimal medium with 0.1% peptone and supplemented with glucose (0.1%), phenanthrene (0.3%) or naphthalene (0.3%). When mycelial cells were gently broken by freezing the mycelium with liquid nitrogen, smashing in a mortar and submitting the samples to an isopycnic sucrose gradients (10–60% sucrose), MO and DHD activities were detected in particular fractions of the gradient, showing specific density values quite different from the density of peroxisomes. Both activities appear to be located in specific compartments different from the peroxisomes.

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# Effect of the pH on the capacity of iron-binding of chaperonin GroEL from *Helicobacter pylori*

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*H. pylori* infection is the chronic bacterial disease more extended around the world. This bacterium colonizes the gastric mucus of the epithelium and during the process secretes proteins such as urease, which helps to support the acid environment of stomach. Another protein also secreted by this pathogen is chaperonin GroEL (HpGroEL). HpGroEL is a protein that has the capacity of binding iron, we believe that this characteristic belongs to HpGroEL because the chaperonin of *E. coli* EcGroEL does not have. Despite of these findings we do not understand what is the objective of iron-binding by HpGroEL. For this reason, we decide to characterize the iron-binding using several hydrogen potential (pH´s). An acid pH broad range was tested because it is well known that *H. pylori* has preference for acid environments. Additionally, pH 7.5 was tested because under this pH is cultivated this pathogen. Interestingly, when the iron-binding of HpGroEL was tested under acid pH close to 40 % remained bound. However, when the experiment was performed using the chaperonin of *E. coli*, no iron-binding was observed, at least to pH 7.5. This experiment (control) corroborated the fact that EcGroEL is not an iron-binding protein. Our overall results clearly show that the chaperonin of *H. pylori* has a particular characteristic of iron-binding under acid and neutral pH´s.

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# Design of aptamers forthe identification of human pathogen bacteria

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Bacterial diseases are considered a health problem because often it is difficult to identify the agent causal. *Streptococcus pneumoniae* is a Gram positive bacterium, which causessevere infectionssuch as pneumonia, meningitis or even worse septicaemia, resulting lethal in several occasions for children and elderly people. The principal treatment is based on antibiotics such as beta-lactamsor inclusive in some cases it is necessary to give antibiotics of third generation like vancomycin. Since the excessive use of antibiotics produces resistance or multi-resistance, it is therefore imperative to develop new alternatives of identification, one of them could be the design of aptamers. Aptamers are small sequences of single DNA close to 60 bases, they can get 3D structure.We believe that 3D structure could be identified foran outer membrane protein of the bacterium. In this work, we decided to identify an aptamer specific for *S. pneumoniae* or *Helicobacter pylori*. We constructed an aptamer library andwe used it to separate an aptamer by cell selex method, which specifically identified *S. pneumoniae* or *H. pylori*. Both sequences were cloned in a plasmid and sequenced in order to revelits identity. This nucleotide sequence was submitted to several *in silico* analysis in order to propose a specific target protein in *S. pneumoniae* or *H. pylori*.

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# Identifying structural determinants of tranglycosidation function in the alpha-amylase enzyme family through residue contact analysis.

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Attaching glucose to some drugs has increased their ability to cross the blood-brain barrier, thus, improving their capability to reach the brain. Additionally, long chain alcohols with a sugar attached to them work as surfactants, which stabilize water-oil mixtures, in the food, drug and cosmetic industries. The alpha-amylase family is a group of enzymes capable of breaking starch into glucose and small maltooligosaccharides through a combination of hydrolysis and sugar transfer (transglycosidation) reactions. In the presence of an alcohol, it has been shown that some of these enzymes are able to attach a glucose unit to the alcohol by means of an alcoholysis reaction, using the inexpensive starch as raw starting material.

In this context, understanding the alcoholysis reaction in the alpha-amylases would increment our control over the addition of glucose moieties to alcohol molecules. As the increment of this reaction has been associated to the augment in the transglycosidation reaction, our aim in this work was to identify structural determinants to increase the transglycosidation reaction.

The approach we decided to follow is based on the analysis of inter-residue contacts, based on the premise that these contacts must be responsible of both structure and function, as these are far more conserved in proteins than either, sequence or structure, and their analysis has allowed to identify function in cases where both, structure and sequence comparison fail to do so. In contrast to current analysis of inter-residue contacts, which use known contact pattern to identify the function of newly diffracted proteins with unknown function, in this work we compared the contacts pattern in the alpha-amylase family, which are proteins with known function, to identify mutation targets that would change the transglycosidation/hydrolysis ratio. We validated this method by evaluating its capacity to classify alpha-amylase members according to their function, as well as its ability to identify negative controls within a group of enzymes and also to identify mutagenic targets already reported to alter the transglycosidation/hydrolysis ratio of an alpha-amylase family member.

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# Following the initial contacts of a mitochondrial protein: the NAC complex and the outer membrane proteins Sam37, Om14 and Tom70

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The vast majority of the mitochondrial proteome is encoded in the nuclear genome and thereby each of these proteins must be synthesized in the cytosol. Mitochondrial proteins must be sorted to the organelle and then translocated into the corresponding mitochondrial subcompartment following a series of steps that together are known as mitochondrial import. A co-translational model explains that some proteins are synthesized and imported in a coordinated manner. In this model, the Nascent-polypeptide Associated Complex (NAC) plays an important role as chaperone for newly synthesized proteins. NAC functions as a heterodimer composed by an $\alpha$ and a $\beta$ subunit, and in the specific case of the budding yeast *Saccharomyces cerevisiae* the $\beta$ subunit is encoded by two different genes. Hence, two different NAC complexes can be formed: $\alpha\beta_1$-NAC and $\alpha\beta_2$-NAC. In our laboratory, we have previously found that the NAC complex has a negative genetic interaction with the genes encoding for the outer mitochondrial membrane proteins, Sam37 and Tom70. In addition it was reported that Om14, another mitochondrial outer membrane protein, is the receptor of the NAC complex in the mitochondria (Lesnik et al. 2014).

In this research we found that there are no genetic interactions between *OM14* and *SAM37*, *TOM70* or the genes encoding for the NAC subunits. In addition, by using the Protein Fragment Complementary Assay (PCA) of Dihydrofolate reductase (DHFR), we studied the physical interactions between the mitochondrial receptors Sam37, Tom70 and Om14 with each subunit of the NAC complex. In these assays, we found that Tom70 does not physically interact with NAC; Sam37 has a physical interaction with $\alpha$ and $\beta_2$-NAC in glucose, whereas Om14 has a physical interaction with $\alpha$, $\beta_1$ and $\beta_2$-NAC in glucose but only with $\beta_1$-NAC in lactate. We are currently investigating if these physical interactions depend on translationally active ribosomes.

Our results indicate that the NAC complex contacts different receptors on the outer membrane, probably depending on the nature of the nascent polypeptide and that it depends also on the metabolic state of the cell.

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# Study of the effect of cytokinin-auxin crosstalk in somatic embryogenesis of *Coffea canephora*

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Cytokinins (CKs) are the plant growth regulators (PGR) that are involved in multiple physiological processes in plants. One less studied aspects is its CK homeostasis (HM). The main genes related to HM are involved in the biosynthesis (IPT), degradation (CKX) and signaling (ARR). In this work we demonstrated the effect of PGR (auxin and cytokinin) in a embryogenic system well established and highly reproducible in *Coffea canephora*, which consists of two stages, one pre-conditioning stages (PCS, 0.52 µM ANA and 2.32 µM KIN) and induction stage (INS, 5 µM BA). The results of transcriptome and qRT-PCR, suggest that auxins (AX) in the PCS are repressing biosynthesis, degradation and signal CKs genes, however, in the INS, there is an increase of genes implicated in CKs perception/signal, indicating perhaps, as in other species, AX are repressing CKs, and CKs are inducing *per se* genes involved in its HM, this is reflected in the endogenous concentration of CKs and the proof crucial is the disrupted of one AX biosynthetic pathway that lead to increase in CK content.

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# Biochemical characterization in vitro of two novel organelar DNA polymerases of plants (POPs) from *Arabidopsis thaliana*.

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DNA replication in both plastids and mitochondria is carried out by DNA polymerases. The genome of the model plant *Arabidopsis thaliana* encodes two DNA polymerases, these have been classified into family the DNA polymerases of plant organelles (POPs): AtPolIA and AtPolIB. In past studies has been demonstrated that double mutation of genes AtPolIA and AtPolIB was lethal, whereas each single mutant showed reduced DNA levels in both plastids and mitochondria. Additionally, the AtPolIB mutant displayed high sensitivity to ciprofloxacin, an inducer of DNA double-strand breaks. These results indicate that two distinct POPs are involved in genome replication for plastids and mitochondria, and that AtPolIB also functions in DNA repair in both organelles. This work is focused on the biochemical characterization in vitro of the two POPs. We are starting with both two synthetic genes modified to overexpress in *E. coli*. We design mutants to the signal localization to plastid and mitochondria and others mutation to evaluate fundamental activities such as polymerization and activity exonuclease, also to evaluate if some of these POPs can polymerize through damaged DNA sites. We found that both AtPolIA as AtPolIB displayed polymerization activity, exonuclease and theses can polymerize through damaged DNA sites. We perform assays for protein crystallization AtPolIBexo$^-$ due to its better overexpression.

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# Involvement of the Bcp1 protein in maturation of the pre-ribosomal 60S subunit, in the ribosomal biogenesis pathway in the yeast *Saccharomyces cerevisiae*.

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The eukaryotic ribosomal biosynthesis pathway is the main activity of the nucleolus and the most tightly regulated process into the cell, where the three RNA polymerases and more than 200 trans-acting shuttling factors are involved. This cellular process begins at the nucleus with the transcription of the rRNA genes and ends with the export of both, large and small pre-ribosomal subunits to the cytoplasm, where these pre-subunits conclude the maturation and the ribosomes are capable to the translation rounds. Defects in any maturation step could generate systemic disorders called ribosomopathies. In the latest events of large pre-ribosomal subunits maturation in the cytoplasm, the SBDS protein in association with the GTPase EFL1 release the ribosome anti-associating factor Tif6 from the 60S pre-subunit. When this mechanism fails, and the releasing of Tif6 is not achieved, the ribosomopathy Shwachman-Diamon Syndrome (SDS) occurs. In our work group, we have preliminary evidences of the involvement of the Bcp1 protein in this cellular stage. Bcp1 (the yeast orthologue of BCCIP) has been initially reported for its participation in DNA repair events and the control of the cell cycle, and therefore for its association with BRCA2 and p21 proteins. Our evidences suggest that Bcp1 is involved in the export and in the latest maturation events of the pre-ribosomal 60S subunits in the cytoplasmic (in association with SBDS and EFL1) in an unknown mechanism, because deficiencies in this protein causes an aberrant accumulation of 60S subunits into the nucleus. Also, Bcp1 cause synthetic lethality with SBDS and EFL1. This work focuses in elucidating the exact role of Bcp1 and its relationship with other transient proteins as SBDS and EFL1, and with an important ribosomal domain called stalk of the large ribosomal subunit, in the cellular context of ribosomal biogenesis.

# HSL esterases in fungi? Bioinformatic and biochemical characterization.

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The heterologous expression and characterization of an esterase from a Basidiomycete fungus is reported for the first time. The *Bjerkandera adusta* esterase (*Ba*EstB) was successfully cloned and expressed using *Pichia pastoris* as expression host. From a detailed structural analysis and according to amino acid similarities and conservation of particular motifs it was established that this enzyme belongs to the α/β hydrolase superfamily, particularly to the Hormone-Sensitive Lipases (HSL) family. The cDNA sequence consisted of a 969 nucleotides, while its genomic counterpart comprised 1133, including three introns of 57, 50 and 57 nucleotides, respectively. Moreover, through three-dimensional modelling studies and phylogenetic analysis we conclude that *Ba*EstB is an ortholog of the previously described *Rm*EstB-HSL of the phylogenetically distant Mucoral fungus *R. miehei*. The purified *Ba*EstB was characterized in terms of its specificity for the hydrolysis of different acyl substrates confirming its low lipolytic activity and a noticeable esterase activity, this in agreement to structural analysis. The biochemical characterization of *Ba*EstB, the DLS analysis and the kinetic parameters determination revealed this enzyme as a true esterase, preferentially found in a dimeric state, displaying good activity under alkaline conditions and relative low temperature (pH=10, 20 °C). Indeed, when comparing this data to data from literature regarding mammal HSL substrate specificity, it may be suggested that fungal HSL proteins are involved in hydrolysis of ergosteroyl esters involved in plasma membrane synthesis. Finally, from a phylogenetic point of view together with our Blastx results, it is suggested that a number of fungal hypothetical proteins could belong to the HSL family

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# Comparative proteomics of amaranth species: Divergence in seed storage proteins and starch synthesis enzymes.

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A third part of world population suffers protein-energy malnutrition and children in developing countries of Asia, Latin America, Near East and Africa, are the most affected, this is because the diet of those regions are based mainly on crops, either cereal or legume, which lack of some essential amino acids; moreover the incorporation of animal foods is very low or practically nil. In the last decades amaranth has been reborn as an alternative crop due to its nutritional characteristics; highlighting the quality of seed storage proteins, because unlike cereals, it is a gluten-free food and has equilibrated essential amino acids profile with high levels of lysine. Furthermore, amaranth plant is resistant to several types of biotic and abiotic stresses. The *Amaranthus* genus is composed by 70 species with high genetic variability among its members; of which only three species are harvested for human consumption, the vast majority are wild species considered as weeds, but these plants could be used for the improvement of domesticated cultivars. So, the aim of this work was to carry out a deep molecular analysis to compare seed storage proteins profiles of wild and domesticated amaranth species. Total seed storage proteins were extracted and analyzed by one dimensional gel electrophoresis (1-DE) and proteome maps were obtained with high resolution 2-DE using 24 cm format. Our results show that each species have diverse isoforms of storage proteins, which impacts on the nutritional quality of seeds; also we observed a particular set of starch synthesis enzymes. Orthologues profiles of granule bound starch synthases were strongly different among species; these enzymes are responsible of amylose synthesis and confer unique starch granules structures. Then, perisperm of seeds were analyzed by scanning electron microscopy and iodine staining, observing important changes of granule starch structures between seed species, that probably implies differences in functionality of amaranth starch. These findings will be important in the implementation of plant breeding programs, focused on the improvement of grain protein nutritional qualities and production of starches with specific physicochemical characteristics.

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# Biophysical properties of a new allelic variant of triosephosphate isomerase found in human prostate cancer cell line.

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In 2012 prostate cancer killed 307,000 people around the world, the next year just in Mexico 5600 men died by the same cause. The allelic variant G233D of human triosephosphate isomerase (HsTIM) was reported in a human prostate cancer cell line (Molecular Genetics, Microbiology and Virology, 2011, Vol. 26, No. 1, pp. 14–20). TIM participates in glycolysis, catalyzing the interconversion of dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde-3-phosphate (GAP). GAP is also a metabolite of the pentose phosphate pathway, which has an essential role in cell division. Moreover, accumulation of DHAP could lead to the non/enzymatic formation of a toxic compound, the methylglyoxal.

In order to investigate the possible role of TIM mutation in cancer development we generated the G233D mutant of HsTIM gene to characterize it biochemically and biophysically. The protein was expressed in soluble form and purified. The kinetic parameters of both senses of the isomerization reaction were determined. It was found that the mutation does not affect the catalytic constants of the reaction in the direction of glycolysis (DHAP to GAP). However G233D mutation decreases the $k_{cat}$ by 4- fold of the reverse reaction. Circular dichroism (CD) spectra in the far UV were obtained to assess the secondary structure content in absence and presence of the weak inhibitor phosphoenol pyruvate (PEP). The mutant exhibited a higher content of alpha helices compared to the wild type (WT). In both variants, PEP increased the content of alpha helices in the same proportion. Intrinsic fluorescence spectra indicated that WT and G233D mutant have a similar tertiary structure. Finally, thermal stability and unfolding reversibility assays were monitored by changes on CD signal at 220 nm, both in presence and absence of PEP. The thermal unfolding transitions indicated that the G233D mutation slightly decreased the Tm app value by 1.5 °C compared to the WT. In presence of PEP, however, the G23D mutant exhibited a Tm value comparable to that of the WT enzyme. Moreover, in both mutants the unfolding cooperativity was significantly improved upon PEP-binding. Under our experimental conditions, the thermal unfolding of both variants was irreversible.

*The in silico* molecular modeling of the G233D mutant provided a rationalization of the interactions in which the side chain of Asp 233 could participate. These interactions include the formation of hydrogen bonds with Lys13, a conserved residue that stabilizes the negatively charged phosphate of substrates. Although our experimental results cannot be extrapolated to the metabolic context of human prostate cancer cells, this study constitutes a first approximation to elucidate the possible role of HsTIM and mutant G233D in human prostate cancer; it also suggests that the mutation could have a positive effect in the energetic balance of the cell, if any, by lowering the isomerization reaction in the sense of DHAP- formation.

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# The Formation of the *Supercomplex* $b_6c$:$caa_3$ when cytochrome $c_{550}$ is Overexpressed in *Bacillus subtilis*.

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INTRODUCTION. *Bacillus subtilis* is a Gram-positive bacterium that has a branched respiratory chain with various dehydrogenases transferring electrons from substrates to the menaquinone-7 pool. Menaquinol can be aerobically oxidized by a branch of various quinol oxidases or by a cytochrome branch composed by the $b_6c$ complex and cytochrome *c* oxidase. This branch includes the membrane-bound cytochromes $c_{550}$ and $c_{551}$ that could be part of the megacomplex $b_6c$: cit $c_{550}$: $caa_3$. The megacomplex has molecular masses between 2000 to 500 kDa and different stoichiometries have been described. Cytochrome $c_{550}$ appears strongly linked to these two complexes and we have found that this union is resistant to the use of detergents and ionic strength. Therefore we are interested in whether this cytochrome is an important element in the organization of the megacomplex.

OBJECTIVE. To over-express cytochrome $c_{550}$ in *B. subtilis* and analyze its influence in the activity and composition of the megacomplex $b_6c$: cit $c_{550}$: $caa_3$ and to find out if it also affects other respiratory complexes.

METHODOLOGY. Three strains were used: WT 168, WT with plasmid pHP13 and WT pHP13-$c_{550}$ (containing the gene for cytochrome $c_{550}$ overexpression). They were grown at 5, 9 and 23 h in a medium with 3% succinate. Membranes were isolated by differential centrifugation. Absorption spectra were made to determine cytochromes content. Activities of the respiratory complexes were measured by oximetry. To view supercomplexes, membranes were solubilized with digitonin and analyzed in clear native electrophoresis. In-gel catalytic activity staining of the respiratory complexes was made to identify modifications in the molecular mass of the respiratory complexes.

RESULTS. Overexpression of $c_{550}$ cytochrome was corroborated in isolated membranes, by the increment in the alpha peak at 550 nm, it was approximately 3 times from the WT. DSLI_PAGE stained for heme also showed the increment on $c_{550}$ that was maximal at 23 hr. Oximetry assays showed that $caa_3$ oxidase activity increased only at 23 h of growth, according to the increment of cytochrome $c_{550}$. We observed an increase in the amount of the supercomplex $b_6c$:$c_{550}$:$caa_3$, and the ATP synthase activity associated to the over-expression of cytochrome $c_{550}$.

CONCLUSIONS. Overexpression of cytochrome $c_{550}$ influences the activities of cytochromes branch and ATP synthase of *B. subtilis.*

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# Effects of cationic molecules on *Candida albicans*

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The effects of different groups of organic compounds on *Candida albicans* were studied; those used were phenotiazine, phenazine, one phenoxazine, acridine, quinoline, and rhodamine derivatives, other diverse cationic compounds, as well as three more with hydrophobic tails. The following parameters were estimated: a) partition coefficients between dichloromethane and water; b) uptake by the cells; c) effects on acidification of the medium; d) efflux of $K^+$; e) uptake of $^{45}Ca^{2+}$ and $^{86}Rb^+$; d) effects on cells growth, and e) effects on respiration. With few exceptions, many produced all the effects, except an inhibition of growth. Regarding the structural requirements, it was found that a) the existence of a positive charge was essential to produce the effects observed; b) in most cases, the charge was provided by one or several amino or imino groups; c) those compounds with methyl or ethyl amino groups were more effective than those with free amino groups; d) the existence of a minimal bulk structure was important, and e) the addition of a lateral chain increased their effectiveness. Among these agents, the most effective found was quinacrine, an old antimalarial agent, which however, did not inhibit growth of the cells (at 60 or 120 µM). Our proposal is that this study may be useful as the basis of future development of antifungals, or perhaps at least one of the compounds tested, quinacrine, to be used in combination with other agents, as already done by other authors.

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# Effect of the flavonoid Morin on the cell cycle in an *in vitro* model of pharynx cancer.

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Head and neck squamous cell carcinoma is a group of different types of cancer in squamous cells in the aerodigestive tract such as mouth, nose and throat. The risk factors for this cancer are tobacco, alcohol and some virus. In 2007, 17.6% of the cases of cancer worldwide was Head and neck squamous cell carcinoma, and Mexico belongs to the first five places in Latin America with this cancer. The treatments could be surgery, chemotherapy and radiation, but only the 50% of patients survive. Because of this, the search for new therapies against this cancer is the principal aim of different groups of Scientifics. The use of *in vitro* models, like FaDu pharynx cancer cells, facilitates the study of different molecules with potential therapeutic use. Flavonoids are compounds that have different biological activities, for example: antioxidant, hepatoprotective, antibacterial, anti-inflammatory and anticancer activity. Morin is a flavonoid found in plants belonging to the family *Moraceae*. There are reports that have demonstrated that this flavonoid is involved in the inhibition of cells transformation and cell cycle progression. The cell cycle is the sum of the processes by which cells replicate. These processes are regulated by a group of proteins called cyclins. The activation of these proteins allows cellular replication. On the other hand, there are also proteins that regulate this cell cycle negatively, like p53 .The aim of this project was to evaluate the cytotoxic effect of Morin in a pharynx cancer FaDu cell line through the regulation of cell cycle progression. This cytotoxic effect was evaluated by an MTT assay. Our results show that Morin has a cytotoxic effect in this cell line in a dose-dependent manner. In addition, employing the wound healing assay, we found that Morin also may have an inhibitory effect in cell migration time dependent. To evaluate the regulation of cell cycle progression, we used Western Blot, and we detected a decrease in a dose-dependent manner in the expression of Cyclin A and B which are proteins related in Phases S and G2 of the cell cycle. With these results we suggest that the cytotoxic effect of Morin in a pharynx cancer FaDu cell line could be because of the down regulation and the arrest in the cell cycle progression.

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# Diversification of the kinetic properties of yeast NADP-glutamate-dehydrogenase isozymes proceeds independently of their evolutionary origin and contributes to the acquisition of fermentative or respiratory lifestyles

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**Summary**

In the yeast *Saccharomyces cerevisiae*, the *ScGDH1* and *ScGDH3* encoded glutamate dehydrogenases (NADP-GDHs) catalyze the synthesis of glutamate from ammonium and α-ketoglutarate (α-KG). Kinetic characterization showed that these enzymes displayed different allosteric properties and respectively high or low rate of α-KG utilization, indicating that the coordinated action of *Sc*Gdh1 and *Sc*Gdh3, regulated balanced α-KG utilization for glutamate biosynthesis under fermentative and respiratory conditions, safeguarding energy provision. Here we have addressed the question of whether there is a correlation between the kinetic properties of the NADP-GDHs, their evolutionary history and the adaptation to the fermentative or respiratory lifestyle. Presented results show that hyperbolic saturation curves and high affinities for α-KG correlate to some extent with acquisition of fermentative metabolism, while cooperativity correlates with respiratory metabolism. *Kluyveromyces lactis* and *Lachancea kluyveri* single NADP-GDH, are similar to either *Sc*Gdh3 or *Sc*Gdh1, which arose from the whole genome duplication event of the *S. cerevisiae* lineage, while *KlGDH1* and *LkGDH1*originated from a *GDH* clade, through an ancient hybridization event that preceded the divergence between the *Saccharomyces* clade and the one containing the genera *Kluyveromyces*, *Lachancea* and *Eremothecium*. Thus, NADP-GDH kinetic properties correlate with fermentative or respiratory metabolism but not with their evolutionary origin.

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# CD43 promotes tumor cells survival, contributing to tumor growth.

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The CD43 sialomucin is a transmembrane protein expressed by cells of the immune system and their transformed counterparts. Recently its expression in non-lymphoid tumor cells such as cervix, colon, breast and lung carcinomas has been described. Overall, CD43 expression by tumor cells is associated to a bad prognosis as CD43 has been described to participate in tumor cell adhesion and motility as well as in the control of cell cycle entry, ultimately favoring cell transformation, tumor formation, and invasiveness. We have shown that the CD43 cooperates with oncogenic signals to promote cell transformation by abrogating the contact inhibition of growth through a molecular mechanism that involves AKT-dependent Merlin phosphorylation and degradation.

To further elucidate the molecular mechanisms through which CD43 participates in cell transformation we took advantage of data from our laboratory showing that in CD43-activated human T lymphocytes the glycolytic enzyme Pyruvate Kinase isoform M2 (PKM2) is tyrosine-phosphorylated (PKM2-$Y^{105}$) and is hence habilitated to perform non-glycolytic functions, namely to function as a protein kinase that activates cell survival pathways. Interestingly, this PKM2 modification is present on different types of cancers and it is considered as a marker of bad prognosis, as is CD43. Thus the aim of this study was to evaluate the participation of the CD43 molecule in the regulation of signaling pathways promoting proliferation and survival of human lung carcinoma A549 cells expressing normal CD43 levels or cells where CD43 expression level was inhibited by RNA interference. Using cellular stress assays (serum deprivation or over-confluent cells), we found that cells with reduced CD43 expression had reduced PKM2 phosphorylated on $S^{37}$ and $Y^{105}$ as well as diminished survival capacities as a result of the modulation of the relative abundance of apoptosis-associated molecules such as Mcl-1 and Bim.

We suggest CD43 can confer benefits to cells such as survival during tumor growth under stress conditions. Accordingly, a better understanding of the cellular mechanisms in which CD43 participates could lead to the identification of new therapeutic targets.

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# Trypsin III Mutant A233N of Monterey sardine (*sagax caerulea*): biochemical characterization

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**ABSTRACT**

Trypsin (EC 3.4.21.4) is the principal member of the serine protease family, and catalyzes the hydrolysis of proteins and peptides specifically at the carboxyl group of lysine and arginine residues. The trypsin III studied from Monterey sardine presented high catalytic efficiency at low temperatures as described for enzymes adapted to extreme cold. Prior to evaluate the kinetics constants of trypsin III mutant A233N we first established the experimental conditions to perform the purification from recombinant overexpression as a fusión protein of trypsinogen and thioredoxin. This strategy resulted in small amount of soluble trypsin. Purification of trypsin III mutant A233N of Monterey sardine was performed by various chromatographic steps, activity and kinetic constants were determined using BApNA as substratre in microplat wells.The mutant A233N showed a band of 45 kDa on SDS PAGE electrophoresis corresponding to trypsinogen – thioredoxin, however due to autoactivation a band of approximately 24 kDa is observed after purification. The mutant presented low activity using the synthetic substrate BApNA with $K_M$ and $k_{cat}$ of 0.2 mM and 00012 $s^{-1}$, respectively. The described protocol allowed us to obtain amounts of soluble protein for further purification and biochemical characterization. Therefore, the trypsin III mutant A233N from Monterey sardine is feasible as a model for structure-function studies for cold-adapted proteins.

# Over-expression, Purification and Crystallization of Novel Homologues of Adenine Glycosylase MutY with [4Fe-4S] Cluster Dispensability.

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The oxidation of guanine to 7,8-dihydro-8-deoxyguanine (Also known as 8-oxo-guanine) is one of the most common DNA lesion caused by reactive oxygen species. During replication an adenine is incorporated across 8-oxo-guanine starting a transversion that is accomplished during a second replication event. MutY is an adenine glycosylase, that belongs to the Base Excision Repair (BER) Pathway, in charge of repairing the initial mismatch 8-oxo-Guanine:Adenine and Guanine:Adenine. This glycosylase is one of the most studied glycosylases and some of its functional defects are related with neurodegenerative and metabolic diseases and cancer. Structurally, MutY can be splited into two major domains an N-terminal domain or catalytic core and a C-terminal domain. The catalytic core, in spite of lacking 125 residues, is active by itself. This domain harbors all the necessary elements for catalysis including two carboxylates and a Phenylalanine involved in base extrusion and hydrolysis, a helix-hairpin-helix motif that stabilizes the DNA-Protein interaction and a [4Fe-4S] Cluster related with DNA lesion search and recognition. Actually, when the [4Fe-4S] cluster is removed the enzymatic activity is completely depleted and some mutation in the [4Fe-4S] motif like those in the cysteinyl ligands alter its kinetic behavior and *in vivo* activity. Thus the function of this cofactor has been found pivotal for MutY activity. However, in our research was identified more than 40 MutY homologues that lack the cysteinyl ligands necessary for [4Fe-4S] coordination. Specially, MutYs from *Entamoeba histolytica* and *Lactobacillus brevis* showed kinetic behavior and *in vivo* activity similar to those enzymes that harbor the cofactor indicating that in some organism evolution has found a way to deal with 8-oxo-guanine with [4Fe-4S] cluster dispensability. In this work we present some results related with the over-expression, purification and crystallization of *Entamoeba histolytica* and *Lactobacillus brevis* MutYs in order to understand the cofactor dispensability in a structural way.

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# Characterization of two mutants in the elongation factor like GTPase (Efl1) and its possible implication in the Shwachman Diamond Syndrome.

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The ribosome is a complex molecular machine essential for protein synthesis. Although the structure and function of ribosomes are well characterized, the molecular details of eukaryotic ribosome assembly remain largely elusive. The biogenesis of ribosomal subunits includes transcription of rRNA, modification of rRNA, rRNA folding, assembly with ribosomal proteins and export to the cytoplasm where the final maturation steps take place. Within this complex process, the GTPase named Elongation factor like-1 (EFL1) together with SBDS release TIF6 that in turn prevents 60S subunit premature association with 40S subunit. Upon binding of Elf1 to the cytoplasmic 60S pre-ribosomes, its GTPase activity is stimulated and it undergoes a conformational change that induces the dissociation of Tif6. This release reaction is assisted by Sdo1, whose orthologue in humans (SBDS), corresponds to the protein mutated in patients with the Shwachman-Diamond-Syndrome. However, there are a 10% of patients that do not present mutations in the *SBDS* gen. In collaboration we have found patients with point mutations in Efl1 causing the same clinical manifestations of SDS.

In this work we investigated the biological consequences of these mutations. Mutations correspond to residues conserved in all EFL1 proteins. *S. cerevisiae efl1Δ* cells are viable when complemented with plasmids bearing the EFL1 mutants. Polisome profile of these cells shows a decrease in the amount of the 80S monosome compared to wild-type cells. Fluorescence microscopy studies demonstrated that TIF6-GFP is re-localized to the cytoplasm in the presence of the EFL1 mutants; despite GTP hydrolysis is not affected. Re-localization of TIF6 has already been observed in *sdo1Δ cells.* To test whether the mutations did not alter EFL1 fold, we produced the recombinant proteins. Circular dichroism and dynamic light scattering studies showed no differences compared to the wild-type protein. This suggests that the overall fold of the protein is intact although atomic changes cannot be ruled out. We believe that the mutants cannot undergo the conformational change necessary to expel TIF6 from the 60S surface.

# Profile genetic evaluation of Th1/Th2/Th17 cytokines in left ventricle of rat during physiological cardiac hypertrophy

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The cytokines play important roles in cardiac remodeling and physiology during pathogenesis of cardiovascular diseases by the identification of immune/inflammatory mechanism in heart failure. Pathological cardiac hypertrophy up-regulate chemokines and pro-inflammatory cytokines, while pro- and anti- inflammatory cytokines increase in hearts of exercise-trained animals. However, the role of cytokines in the physiological cardiac hypertrophy induced by pregnancy have not been examined. Therefore, the aim of the present study was to evaluate the profile genetic of Th1/Th2/Th17 cytokines in left ventricle of rat during pregnancy. The mRNAs level for interleukin (IL) -6, IL-17b, IL-1b, IL-10, IL-4, interferon gamma, tumor necrosis factor (TNF) -alpha and, TNF-beta increased during pregnancy compare to non-pregnancy. Our results show that physiological cardiac hypertrophy induced by pregnancy up-regulate pro- and anti- inflammatory cytokines suggesting compensatory response to cardiac remodeling and physiology.

# "Structural study of the extended spectrum β-lactamase TLA-1 by X-ray crystallography"

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β-lactam antibiotics are bactericidal agents able to hinder bacterial cell wall synthesis via the inhibition of the penicillin binding protein (PBP) and causing, as a result, its lysis. As a response bacteria have developed different defense mechanisms, being the production of β-lactamases the predominant cause of resistance. These enzymes catalyze the hydrolysis of the β-lactam ring avoiding the binding of the antibiotics with the PBP. β lactamases are divided in four major classes, being class A the largest. Class A β-lactamases have four characteristic motives on their active site which are; $S^{70}$-X-X $K^{73}$, $S^{130}$-X-$N^{132}$, $K^{234}$-$T^{235}$/$S^{234}$-$G^{235}$ and the Ω-loop distinguished by, the conservation of the $Glu^{166}$ and a *cis* conformation on the peptide bond between the $Glu^{166}$ and the residue 167. Inside class A there are members able to confer resistance to penicillin and; first, second, third and fourth generation cephalosporins and are inhibited by clavulanic acid these are called extended spectrum beta lactamases (ESBL). In the year 2000 Silva characterized a novel ESBL, produced by an isolate of *Escherichia coli* found in a hospital in Mexico city, this novel ESBL was designated as TLA.-1 This enzyme is a member of the family of PER-like β-lactamases, this family is characterized by a *trans* conformation of the Ω-loop, which enables a better arrangement of bulkier antibiotics, such as cephalosporins, in the active site.

In recent years TLA-1 producing microorganisms have been found in several hospitals in Mexico. Also, in El Salvador and Peru, genetic sequences of putative β-lactamases with an identity of over 94% with TLA-1 have been reported increasing the medical importance of this ESBL. In this study we will determine the crystallographic structure of the extended spectrum β-lactamase TLA-1 of *E. coli* with the purpose to stablish the structural differences and similarities between TLA-1 and other ESBLs. To achieve this TLA-1 will be overexpressed with different strains of *E. coli* using the expression vector pJ411 and the protein will be purified using two chromatography columns, first a cation exchange column, followed by a molecular exclusion column. Once pure, crystallization trials will be placed using microbatch method. Then, experimental data will be obtained via X-ray diffraction to determine the structure. After this, the structure will be analyzed and compared with other ESBLs. At the current moment, he expression protocol and a partial purification of TLA-1 has been accomplished.

***The enzymes of the arginine metabolism and its activity in red blood cells.***

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*The metabolism of L-arginine involves competition for the amino acid of three metabolic pathways: the arginase pathway, involved in proliferative processes, inflammatory, and regenerative; the route of nitric oxide synthase (NOS) involved in the activation of the inflammatory and cytotoxic defense; and the route of arginine decarboxylase* **[1]** Arginase activity is involved in cytotoxicity on normal and cancer cells, or on microorganisms; this enzyme is induced by TH1 cytokines (IL-4, IL-10 and IL-13) and lipopolysaccharide of bacterial wall (LPS) **[2]**; Arginase competes for the L-arginine with the inducible nitric oxide synthase (iNOS), demonstrating its importance in immune and inflammatory response **[2]**; The metabolism of this amino acid results in the synthesis of a number of essential compounds in the immune response, which have a fine regulation on the different pathways **[2]**. For example, the N (ω) -hydroxy-L-arginine (NOHA), product of iNOS activity, has an inhibitory activity on arginase ***[2].*** *Arginase is also part of the urea cycle and produces L-ornithine and urea. Urea is involved in detoxifyication of the body amino nitrogen* ***[2]*** *and in the inhibition of the NOS activity* ***[2].*** L-ornithine that was obtained may follow three paths, one from enzyme ornithine aminotransferase (OAT) to L-proline, which is involved in the repair and maintenance of muscles and bones; another one from glutamic acid, which produces GABA through decarboxylation **[1]**. Another path that the ornithine metabolism may follow is through the activity of the decarboxylase (ODC) which synthesizes polyamines; polyamines are important for cell division and immunosuppression, can regulate the function of some ion channel and inhibit NOS activity **[2]**. Finally ornithine can react with carbamyl phosphate and produce citrulline through the ornithine transcabamilase (OTC) enzymatic activity **[1]**. The arginine decarboxylase pathway produces agmantine **[2]**. Endogenous agmantine is induced in response to stress and / or inflammation; it is a neuronal neuroregulador and inhibitor iNOS and, in irreversible way of nNOS. Polyamines can also be synthesized in this path from agmantine, through the action of agmantinase. **[2]**. Many of the compounds synthesized in the metabolism of arginine are cell-signalling molecules: NO, agmatine, citrulline, glutamate, GABA and polyamines **[2].** Thus, the arginine metabolism and its regulation are of considerable physiological significance. If we focus on *red blood cells* (RBC), its maturation is associated with the loss of enzymatic functions. For this reason, it has been proposed that the RBC are metabolically trivial and their role is limited to transport gases and nutrients to the cells. However, the activity of type-I arginase was found in RBC from primates **[1]**. Comparably the presence of NOS in RBC has been a matter of controversy for some authors **[1]**. Neverless, it has been shown that RBC not only act as “NO sinks,” but also exert an erythrocrine function by synthesizing, transporting, and releasing NO metabolic products and ATP, controlling systemic NO bioavailability and vascular tone. Hemoglobin plays a central role in these biochemical processes. We are interested in determining whether the enzymatic activities associated with the metabolism of arginine (arginine metabolic route) are present in RBC, by looking at the existence of the products formed as result of its enzymatic activities, both spectrophotometrically and by radiolabelling, and determining the effect of different inhibitors on the enzyme activity involved.

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# *OlsF* expression in *Burkholderia andropogonis* increase environmental stress tolerance

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The genus *Burkholderia* contains large number of diverse species which include many clinically important bacteria, phytopathogens, as well as environmental species. Among species that belong to genus *Bukholderia* that can induce plant diseases is the well known pathogenic bacterium *B. andropogonis*, which affect more than 52 species of 15 families of unrelated monocotyledonous and dicotyledonous plants. Also, *B. andropogonis* has an atipic polar lipid profile , as it synthesizes two forms of phostatidyletanolamine (PE) and hydroxylated (PE-OH), but it lacks ornithine lipids (OL) and hydroxylated (OL-OH), commons for the genus *Burkholderia*. We expressed *olsF* gene from *Serratia proteomaculans* (involved in OL synthesis) in *B. andropogonis LMG2129* strain. We observed changes in the membrane lipid profile, forming two new lipids, modified and unmodified OLs, and also absence of PE-OH naturally synthesized for *B. andropogonis*. With the purpose to identify the role of OLs in *LMG2129* strain to different environmental stresses and their possible effect in phytopathogenicity, wild-type and transformant strain *LMG2129* were grown at different temperatures (20-42 °C). It was observed that strain expressing *olsF* gene was able to grow in temperatures up to 42 °C, compared to the wild-type strain which only grows at 30°C. The temperature resistance in the *LMG2129* strain could be due to the modified or unmodified OLs formed in the membrane. Presently, we characterize the OLs function in environmental stress resistance as acidic pH and antibiotic tolerance, as well as pathogenicity tests in diverse hosts.

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# Silencing of the HIF-1α affect the glucose and lactate concentrations, and G6PDH activity in shrimp infected with the WSSV

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The Warburg effect is an abnormal glycolysis response that is associated with the white spot syndrome virus (WSSV) infection in shrimp. The hypoxia inducible factor 1α (HIF-1α) is a transcription factor regulator of cell metabolism and cellular adaptation to low oxygen stress. Have been demonstrated that HIF-1α regulate anaerobic glycolysis in shrimp exposure to hypoxia. The HIF-1α function under Warburg effect in shrimp infected with the WSSV is not defined. Therefore, the aim of the present study was to evaluate the glucose and lactate concentrations, and glucose-6-phosphate dehydrogenase (G6PDH) activity WSSV in HIF-1α knockout shrimp. Glucose and lactate concentrations increased 2- and 3- fold, respectively, in gills from shrimp infected with WSSV, while in HIF-1α knockout shrimp the concentrations were maintained as controls. Interestedly, glucose and lactate concentration decreased 2-fold, respectively, in muscle from shrimp infected with WSSV and maintained as control in HIF-1α knockout shrimp. The G6PDH activity increased 2-fold in gills from shrimp with WSSV infection compare to control, and decreased 2-fold in muscle. In contrast, G6PDH activity increased 3- and 2-fold in gills and muscle, respectively, from HIF-1α knockout shrimp compare to control and WSSV infections. Our results demonstrate that silencing of HIF-1α affects of tissue-specific manner the Warburg effect, giving insights of the action of HIF-1α in these response.

# Energetics basis of allosteric inhibition of ABL tyrosine kinase involved in chronic myeloid leukaemia.

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Under normal conditions, the catalytic activity of the tyrosine kinase Abl is tightly regulated by various auto-inhibitory mechanisms. In chronic myeloid leukaemia (CML), Abl is constitutively active through genetic fusion with Bcr. This malignant hematopoietic disease affects around 1.5 millions of people patients worldwide. Expression of Bcr-Abl protein leads to activity deregulation of the tyrosine kinase due to the loss of an N-terminal inhibitory element present in normal Abl. Imatinib is a phenylaminopyrimidine derivative that has high affinity and specificity for the catalytic site of Abl, which has proved clinically successful in the treatment of CML. However, due to imatinib resistance by point mutations in the Abl kinase domain, more than 100 new several inhibitors have been further developed, such as dasatinib, although none of them shows efficiency in all treated patients. All of these approved drugs compete with ATP for the binding site of Bcr–Abl and provide significant clinical benefit. However, acquired resistance has become a major challenge for treatment of CML. An alternative strategy to discovery of new Bcr–Abl inhibitors involves the development of agents targeting Bcr–Abl sites which are different with ATP site. Myristoylation at myristoyl binding pocket (allosteric site) of Bcr–Abl could stabilize kinase in its inactive conformation. It is expected that small molecules which bind to the myristoyl site could keep Bcr–Abl in inactive configuration. GNF-2 was demonstrated to bind to the myristoyl pocket which located near the carboxy terminus of ATP site. It could bind to myristoyl site and display potent inhibitory activity against both wild and mutant Bcr–Abl. in order to obtain information on the structural dynamics of myristoyl-binding site in this work we characterized the binding of fatty acids to myristoyl-binding site of Bcr-Abl kinase domain by isothermal titration calorimetry (ITC) at different temperatures. The binding heat capacity ($\Delta Cp$) was determined for different fatty acids tritiated. The results showed the energetics behavior that govern the allosteric inhibition of Abl kinase and widely discussed in the poster session.

# Study of the physical interaction between the essential GTPases Gpn1 and Gpn3.

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Gpn1 and Gpn3 belong to the GPN family of GTPases, which received this name due to an invariable Gly-Pro-Asn motif in their amino acid sequence. They are essential proteins and are present in archaea and eukaryotic cells. Gpn1 and Gpn3 are required for the nuclear localization of RNA polymerase II, the enzyme that transcribes all protein coding genes. Crystallographic structures from *Pyrococcus abyssi* and *Saccharomyces cerevisiae* (1YR6 and 5HCI pdb codes respectively) show a homodimeric assembly of Gpn1. Recently, we demonstrated that human Gpn1 and Gpn3 retain each other in the cytoplasm of HEK293T cells, presumably by a direct interaction between these two proteins. In this study, we demonstrated by FRET that there is a direct physical interaction between Gpn1 and Gpn3. Interestingly, we found that the W132D mutation in Gpn1 disrupts the FRET signal. These results open an avenue of research to evaluate the functional importance of Gpn1-Gpn3 interaction on the cellular functions described for these GTPases.

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# Determination of Kinetic Parameters of Trypsin I from Pyloric Caeca of Monterey Sardine (*Sardinops sagax caerulea*) Using Isothermal Titration Calorimetry

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Pyloric caeca of Monterey sardine (*Sardinops sagax caerulea*) shows an expression of trypsin I according to a cDNA characterization, is a psychrophilic enzyme according to the catalytic efficiency (*kcat*/*KM*) obtained by spectrophotometric essays. This parameters can be obtained by Isothermal titration calorimetry (ITC) using thermal power generated by the enzymatic conversion of substrate to product; were the rate of reaction is directly proportional to thermal power. The objective of this study was to obtain kinetic parameters of trypsin I at different temperatures using ITC. To reach the objective, the enzyme was purified from the pyloric caeca of the sardine using molecular exclusion and affinity chromatography obtaining a yield of 1.0 mg/mL. At 4 °C *kcat and KM* of Trypsin I were 4.4 s-1 and 0.3 µM respectively. At 10°C were 3.05 s-1 and 0.2 µM (*kcat and KM)* and at 15°C *kcat* was 4.93 s-1 and *KM* 0.7 µM. At 20°C and 30 °C no adjust was possible to make, nonetheless at 25 °C a *kcat and KM of* 5.30 s-1 0.2 µM were obtained. The higher values of *kcat* were for 15 and 25°C, values of *KM* were similar at 4, 10 and 25°C. The catalytic efficiency (*kcat*/*KM*) were 13 , 15, 7 and 25 s-1 µM-1 at 4, 10, 15 and 25°C respectively, showing a better catalytic efficiency at 4°C than 15°C; and 3 times fold at 25°C. With this results we can reassert the psychrophilic behavior of trypsin as it´s *kcat*/*KM* is higher than mammalian trypsins. The kinetic behavior of enzymes leads to understand biochemical pathways and catalytic mechanisms; were ITC provides a universal approach to determining the kinetic behavior of enzymes.

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# EVALUATING AN ASSESSMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

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This paper brings together the data obtained from the answers given by the students who presented the second final test of Biochemistry and Molecular Biology at the Faculty of Medicine of the UNAM recently, with the objective of evaluating the reagents used. The assessment tool was applied to 613 students. The study was observational, cross-sectional, descriptive and retrospective. A statistical analysis was performed using 4 techniques. 1) Index of difficulty (Pi), 2) Discrimination index (Di), 3) Discrimination coefficient ($r_{pbis}$), 4) Alpha of Cronbach. Pi indicates whether a question is very easy or very difficult; Di indicates if the test and a question measuring the same skill or competence; $r_{pbis}$, helps to know if more suitable people are that obtained the correct answers; Cronbach's alpha indicates the reliability of the test. The evaluation instrument consisted of reactive 80 multiple choice and the database is exported to Excel, then processed with the statistical package SPSS. The objective of this work was the questions that met at least three of the four studied techniques to identify in this case shall be considered suitable to be part of a Bank of reagents. The evaluated topics are: water and pH, structure of proteins, enzymes, chemical, oxidative phosphorylation and metabolism of carbohydrates, chemistry and lipid metabolism hormones and genetics. Reagents 80s only 25 (31%) covered with the acceptance criterion. The topics that the reagents were acceptable: water and pH (75%), oxidative phosphorylation (40%), carbohydrates (58%); on the other hand, on the issues of genetics, enzymes and proteins very few reagents were considered to be acceptable. With the obtained results it is concluded that the people who answered the test was decisive for this study, since only 46% earned a passing grade, in addition to developing reagents are careful among others, writing, content, relevance, clarity and that the answers are clear.

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# Induction, purification and biochemical characterization of a pectinase from *Ophiostoma piceae*

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*Ophiostoma piceae* an ascomycete ophiostomatoid is a fungus saprobe that is dispersed by bark beetles to pine, fir, elm, hemlock, etc. In trees, the fungi localize it in the sapwood of logs and lumber, where is present the pectin.
Pectin is the first carbon source used by microorganisms in the stage of colonization in plants. The cell wall can be degraded by enzymes secreted by fungi, among which the pectinases, group of enzymes with different substrate specificity, are responsible for degrading different structures of pectin.
In *Ophiostoma piceae* complex (*O. ulmi* and *O. novo-ulmi*) has been reported the production of these enzymes without a correlation with virulence.
Analysis *in silico* of genome and transcriptome from *O. piceae* show the existence at least one gene encoding a pectinase. It was reported likely when the fungus is grown in a medium with sawdust. However, a date, there are any biochemical and physiological studies about these enzymes in *O. piceae,* a reason that motivated us to focus on the study of them, principally into induction, purification, and biochemical characterization.
Induction of pectinolytic complex was performed in minimal medium Mathur plus 0.2% glucose, using pectin and bean stubble as inductors, over a period of 11 days. The enzyme activity was determined using the DNS method of Miller (1959). Homogeneity purification was achieved using different resins (adsorption and molecular size exclusion) and confirmed with 2D-SDS-PAGE.
We recover two proteins with an apparent MW of 128.9 and 59.3 kDa and pI values of 5.2 and 4.6 respectively with a possible homodimeric conformation. Both enzymes display activity at acidic pH, are independent of divalent cations, however, are strongly inhibited by EGTA. By TLC, we observed only monosaccharides as a product of hydrolysis with different pectins, suggesting the enzymes are polygalacturonases or pectin esterases.

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# Protocol of expression and purification of recombinant mitochondrial DNA Polymerase from *Saccharomyces cerevisiae*

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DNA-Polymerase MIP1, from *S. cerevisiae,* is an enzyme responsible of mitochondrial genome replication. MIP1 has the ability to be processive by itself, because synthesizes fragments up to 6 kb,in contrast with POLG (Human mitochondrial DNA Polymerase) and the DNA Polymerase from T7, requires a processivity factor (p55 and Thioredoxin) to increase the length of the stranded DNA. In past studies has been observed that this DNA Polymerase is capable to replicate through DNA damage by oxidants agents, alquilants and occurred with UV. In this work, a one mutant of MIP was realized. This mutant has deletion over 196 aminoacids from the C-termini region. Then, this mutant was cloned into pET 19-b pps vector and finally overexpressed. The protein (MIP1 mutant) was purified by immobilized metal affinity chromatography, ionic exchange and finally by size-exclusion chromatography. The protein was obtained with 95 % purity, and crystallization assays were tried in specifics conditions for proteins binding DNA.

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# *Yersinia pseudotuberculosis* OppA protein chaperone activity on the α-glucosidase enzyme

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The *Yersinia pseudotuberculosis* YPIII peptide binding protein OppA is the substrate binding protein (SBP) from the oligopeptide transporter (Opp). This system is a five-component ABC transporter composed by the following proteins: the periplasmic SBP OppA, the permeases OppB and OppC, and the ATP-ases OppD and OppF. The Opp system is related to the incorporation of nutrients, virulence and antibiotic susceptibility. Other important proteins in the periplasm are the periplasmic chaperones, which are involved in the folding in novo of proteins, retrieve de native conformation of proteins denatured by stress, assemble oligomers and support in protein degradation.
Some periplasmic chaperones have dual activity as SurA, PpiD, and FkpA which also act as peptidyl-prolyl *cis*-t*rans* isomerases, and DegP (HtrA) that has protease activity. In addition to the above mentioneted periplasmic chaperones has been reported in *E.* coli that SBPs MalE and OppA have chaperone-like activity. The purpose of this work is to demostrate the chaperone activity of OppA protein of *Y. pseudotuberculosis* on the α-glucosidase enzyme denatured by urea. So the OppA protein was purified by Ni-NTA, testing of chaperone activity was carried out under the following conditions: one unit of α-glucosidase was denatured with urea 3.75 M, 20$^{o}$C, after 40 minutes were added the chaperones: albumin 1.15 µM (used as negative control), OppA 34 µM and HtrA 21 µM (positive control) , in a final volume of 500 µL, and incubated during 90 minutes at room temperature and subsequently added 2 µg maltose, after 90 minutes the glucose concentration was measured with the glucose oxidase method. The tests were performed in triplicate. The rate of recovery of activity in renaturation buffer $KH_2PO_4$ 50mM and KCl 200 mM was 36%, with albumin in renaturation buffer 55%, with OppA 83%, and with HtrA is the 93%. The net recovery of activity with albumin was 18%, with HtrA 57 % and with OppA is 47%. With the data obtained we conclude that OppA protein has chaperone-like activity on α-glucosidase under these conditions.

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# Differential protein expression in gastric premalignant lesions and gastric cancer by iTRAQ labeling-based proteomics approach

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Gastric cancer is still a major cause of cancer mortality in our country, so it remains a serious problem of public health. Among the problems in the managing this disease are the late diagnosis and limitations of medical-surgical therapies available. In principle it is possible to prevent gastric cancer as it is preceded by a long latency period [1]. The knowledge of premalignant conditions, such as intestinal metaplasia (IM) and dysplasia can be considered as ideal stage for the early detection of gastric cancer [2]. However, these lesions have a poor prognosis power; therefore, so far, it is difficult to predict the possible progression to cancer from IM to GC. Only few biomarkers are currently available for the detection and prognostic evaluation of gastric cancer. These include circulating antigen markers of gastrointestinal malignancies, (CA 72.4, CEA and CA19.9) [3]. However, their sensitivity and specificity does not allow recommending them as diagnostic tests for gastric cancer. Our work aimed to study the expression profile of proteins in biopsies of patients with intestinal metaplasia (colonic type) and gastric cancer (intestinal type), to identify specific proteins that potentially could be used in the future as a biomarkers associated with the malignant transformation, to help doctors identify patients at risk of developing intestinal metaplasia and gastric cancer.

We select 4 biopsies from subjects with each of the following outcomes: non atrophic gastritis, colonic type of intestinal metaplasia and intestinal type of gastric cancer. The protein from the 12 biopsies was isolated with urea buffer, after the trypsin digestion the four samples of every pathology were mixed, the three pooled samples obtained were divided into four aliquots in order to have four replicates, then every aliquot were labeling with a different iTRAQ reagent, and then mixed to obtain two pooled samples, each was separated on reversed-phase liquid chromatography (RP-LC) system, to obtain 15 fractions of each pool. Mass spectrometry analysis was carried out on a Q Exactive Hybrid Quadrupole-Orbitrap, and analyzed with Max quant software. We find some potential protein biomarkers that can be used as gastric cancer predictors.

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# Spectroscopic characterization of metal binding to AtLEA4-5 peptides.

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The proteins known as Late Embryogenesis Abundant (LEA), are intrinsically unfolded, and their expression and accumulation in plants is increased under hydric stress caused by abiotic factors or physiological conditions. Particularly, the group 4, from Arabidopsis thaliana (AtLEA4), consists on three basic and hydrophilic proteins, AtLEA4-1, AtLEA4-2 and AtLEA4-5, which have amino terminus motifs highly conserved. Also, it has been suggested that the binding of metals to LEAs could prevent cellular damage caused by oxidative stress. In this project we are studying the metal ion binding to the AtLEA4-5 using model peptides, different spectroscopic techniques, and isothermal titration calorimetry. So far, we have found that the protein is able to bind Ni(II), Zn(II) and Cu(II) with moderated affinity. In the case of Cu(II), there are at least three different binding sites with coordination modes of 4 nitrogens, and 3 oxygens and one nitrogen.

# Enzymatic synthesis, structural and functional characterization of Primase-polimerase (PRIM-POL) of *Arabidopsis thaliana*

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Mitochondria and plastids are eukaryotic organelles that posses their own genomes. Organellar genomes replication include a primase which synthesize an initial oligoribonucleotide primer and a DNA polymerase which elongate the primer. Recently, it has been identified DNA primase-polymerase protein (PRIM-POL) specialized in translesion DNA synthesis and DNA replication in human cells. PRIM-POL is a DNA primase polymerase of the archaeo- eukaryotic primase (AEP) superfamily. Human PRIM-POL is localized in both the nucleus and mitochondrion, where it is involved in translesion synthesis bypass, including UV photoproducts and (re)-priming properties. Although recent studies have established the importance of PRIM-POL in damage tolerance during DNA replication in cellular and animal model systems, through alignment, we identify a potential homologue PRIM-POL in plats. The replication has been poorly studied in chloroplast. The purpose of this work is to elucidate the biological importance of the presence of a polymerase primase translesion synthesis in plants.

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# *In vitro* evaluation of the elongases (ELOVL 1-7) expression on insulin sensible and resistant adipocytes.

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## Abstract

Obesity is a global problem associated with metabolic disorders and the development of chronic diseases such as type-2 diabetes, cardiovascular disease, hypertension, cancer and others. Adipose tissue, commonly seen as a simple lipid deposition, now is recognized as an important endocrine tissue that regulates metabolic balance by secretion of a variety of signals. White adipose tissue is directly involved in obesity and associated with pathological conditions such as insulin resistance (IR). Adipogenesis and IR are processes that occur by activation of a coordinated gene expression program, which results in changes of both, the activity and the amount, of key proteins in the physiology of the adipocyte, primarily in lipid and glucose homeostasis. The elongation process is important in the metabolism and is currently investigated in the development of diseases such as IR, cancer and obesity. The present study evaluated the role of ELOVL in adipogenesis and IR in a cellular model of pre-adipocytes. 3T3-L1 cells were differentiated using insulin, biotin and IBMX treatments for 7 days; and RI was induced by treating the cells with TNF-α. RNA was extracted every 24 hours during the 7 days of differentiation and RT-qPCR was performed in order to analyze gene expression ELOVL (1-7) and adipogenesis markers associated with IR. The results indicated increased gene expression ELOVL (1-7) in differentiated adipocytes, difference was more remarkable on days 1, 3 and 6. ELOVL-7 was overexpressed at the first day of differentiation and then decreased its expression. In the same way, ELOVL-1, ELOVL-4 and ELOVL-6 were overexpressed during days 1, 3 and 6 of differentiation, with clear reduction was observed on days 2, 4 and 5. ELOVL-5 presented an increase on days 3 and 6. Moreover, at the end of the differentiation process, markers of adipogenesis (lipin, NF-kB, PPARα and PPARγ) were overexpressed while CRBP1 and SRBP1c showed no change. Furthermore in the IR model, ELOVL-4 and ELOVL-5 were overexpressed on day 3, and only ELOVL-5 maintained the overexpression on days 5 and 6. In the same way, only PPARy was overexpressed and no overexpression was observed for PPARa and CREBP1. These results give a better understanding of the role of these enzymes in the pathophysiology of insulin resistance.

**Key words:** adipogenesis, ELOVL, insulin resistance.

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# Expression and purification the plant-specific ssDNA binding proteins: mtSSBs, OSBs and Why, from *Arabidopsis thaliana*.

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The ssDNA binding proteins, SSBs, play a role as stabilizers of ssDNA in the replication process. In *Arabidopsis thaliana* has been identified a couple of SSBs encoded in the nuclear genome, AtSSB1 and AtSSB2. It was observed that both are located in chloroplast and mitochondria. AtSSB1 and AtSSB2 bind to ssDNA, but not dsDNA. Furthermore, the organellar ssDNA binding proteins (OSBs) comprise the second class of SSBs in plants. These OSBs have a similar domain to SSBs, but additionally have either one, two or three PDF motifs in the C-terminal region responsible for binding to ssDNA. *A. thaliana* also encoded in its nuclear genome four OSBs: AtOSB1 and AtOSB2 that are localized in the mitochondria and chloroplasts, respectively, while AtOSB3 is located in chloroplast and mitochondria; and AtOSB4 only is known that binds to ssDNA. The Whirlys are a third group of ssDNA binding proteins, which are associated with breaking dsDNA repair. *A. thaliana* encodes four Whirlys: Why 1, Why 2, Why3 and Why 4.

In this work the following genes were cloning: At SSB1, AtSSB2 and AtOSB1 were expressed into pCri1b vector; AtOSB2 and AtOSB3 into pET19b-pps; and Why2 into pET28b-pps; then were over-expressed and purified by different techniques each one of these six proteins, obtaining them in soluble form with different yields. The AtSSB2 showed the best purification yield, so it was chosen for crystallization assays.

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# Functional complementation of the ribosomal EFL1 GTPase domains

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Ribosomes are ribonucleoproteic complexes specialized in protein synthesis. In *Saccharomyces cerevisiae*, the ribosome is composed of two subunits known as the minor (40S) and large (60S) subunits. Each subunit has different composition and function: the 40S subunit decodes the information contained in the mRNA and it is formed by the 18S rRNA and 33 proteins, while the 60S subunit forms the peptide bond during protein synthesis and consists of the rRNA 25S, 5.8S y 5S, and 46 proteins. The process to produce ribosomes is known as ribosome biogenesis and involves synthesis, assemble, processing and export of the aforementioned biomolecules. Maturation of the 60S subunit in the cytoplasm requires the assistance of two accessory proteins; the GTPase Efl1 and Sdo1, to promote the release of Tif6 form the 60S subunit and functional ribosome assembly. Efl1 is homologous to the EF-2 translocase with a G-domain and four additional domains numbered from II-V. The function of these domains is not known except for that of the G-domain responsible for GTP hydrolysis.

The *EFL1* gen is essential for the viability of *S. cerevisiae* cells. We used this property to assess the importance of each domain of EFL1 and combinations thereof by genetic complementation in yeast *efl1Δ* cells. Positive complementation was evaluated by the recovery of living cells. These experiments demonstrated that all the domains of EFL1 are essential for protein function. Protein expression was confirmed by western blot. Additionally, we built inter-species EFL1 chimeras consisting of *S. cerevisiae* domains 1+2 together with *H. sapiens* domains 3-5 and *H. sapiens* domains 1+2 fused to *S. cerevisiae* domains 3-5. We were able to recover viable progeny only when testing the complementation with the EFL1 chimera containing domains 1+2 of yeast. This suggests that the recognition of these domains with the ribosome are specie specific.

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# Expression of recombinant Pearlin of *Pinctada fucata* in *Escherichia coli*

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**Keywords:** Pearlin, Biomineralization, Protein Recombinant

Nowadays, a variety of biominerals are found distributed in nature, including the shells of mollusks. This is composed of two layers known as prismatic layer that bears calcite and nacreous layer bearing aragonite. These layers contain about 5% organic matrix, which consists of proteins, carbohydrates and lipids, this proteins are considered to play an important role in the crystal nucleation, crystal growth and are believed to provide the organic matrix framework around which the calcium carbonate is deposited (Marin & Luquet).

Biomineralization research on mollusk shell has mostly focused on nacre formation due to its high commercial value, mainly proteins on the organic matrix, among them Pearlin (Zhang & Zhang).

The Pearlin is a structural protein to be a highly conserved among members of genus *Pinctada*, its molecular weight is approximately 15 kDa and it is a protein rich in glycine, tyrosine, cysteine, asparagine, aspartic acid and arginine. Experiments of PAS and Alcian blue showed that Pearlin is a sulfated glycoprotein, because of contains sulfate groups bound to mucopolysaccharides on tyrosine residues or cysteine, also has six sites N- myristoylation in glycine residues located at the amino terminal, whose function is participates in the formation of the organic matrix framework around which the calcium carbonate is deposited. Pearlin function as a template for nucleation, but its function and molecular mechanism still being investigated (Miyashita *et al*., 2001).

As first step, the gene encoding Pearlin by *P. fucata* was cloned into the pJexpress414 vector (DNA 2.0). The construction was transformed into *E. coli* cells chimiocompetents ER2566. The first expression test were performed on cultures 30 ml, then we determined the conditions for induction. These cultures were induced with IPTG 1 mM to achieve a $OD_{600}$ between 0.5-0.6, for 5 hours at 37 °C under stirring. Monitoring the growth of bacteria by changes in the optical density at intervals for one hour. Finally, the cells were harvested by ultra-centrifugation and lysed by sonication. After analysis of crude extracts (soluble and insoluble fraction) by SDS-PAGE 15% we observed an enriched band about 18.5 kDa in the insoluble fraction. Finally we confirming the expression of Pearlin in *E. coli*, a SDS-PAGE 15% was performed and analyzed by mass spectrometry.

Once we have obtained the purified protein, we will proceed to crystallization and determination of crystallographic structure.

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# Biochemical characterization of DARPins with a single binding module.

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Designed ankyrin repeat proteins (DARPins) were succesfully created as new binding proteins with remarkable advantages in comparison to antibodies. DARPins were derived from natural ankyrin repeat (AR) proteins by consensus design. DARPins scaffold consists of two capping repeats that surround one or more randomized binding modules of 33 aminoacid residues. Each module forms a β-turn followed by two antiparallel helices and a loop reaching the β- turn of the next repeat. There is no limit to the number of repeats that can fold in natural AR proteins. Although, most of them have from four to six repeats, it has been reported some AR proteins with twelve repeats in a single domain. Some of the advantages of these proteins include high expression levels as soluble proteins in the cytoplasm of *Escherichia coli* and good purification yields in amounts up to 100 mg per 1L of bacterial culture. Also, DARPins are extremely stable with Tm values around 70 ºC and this stability is proportional to the number of binding modules. Several studies have revealed a great diversity of applications of DARPins recognizing a multitude of protein targets with different properties. The specificity of the DARPins is due to seven variable positions located at the binding module. In this study, we obtained a library of DARPins with a single module and chose five in order to characterize them at biochemical and structural level. All of the selected DARPins showed improved expression levels and were purified by immobilized-metal affinity chromatography with high purification yields. Spectroscopic studies revealed the typical structure previously reported for this kind of proteins and their thermostability was followed by dichroism circular signal at 222 nm showing Tm values in a range of 55-60 ºC. Some preliminary studies of interaction of these DARPins against specific targets were shown.

**NMR dynamic basis of the EphA-SAM and Ship2-SAM complexes**

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The Eph receptor is Tyrosine kinase family that interact with their ligand Ephrins, play a key role in the signaling processes for the cell-cell interaction and cell migration involved in the spatial organization, embryogenesis, tissue patterning and blood vessels formation. The anomalous regulation of this receptor have been related to several pathological conditions such as cancer. In the cytoplasmic region of this transmembrane receptor, the C-terminal region is the Sterile Alpha Motif (SAM) domain. This highly conserved SAM domain in the Eph receptors is known to have an important function in the signaling process, modulating the protein interactions. The SAM domain can form several homotypic and heterotypic protein complexes, regulates the receptor clustering formation as well as the complex with diverse proteins, including the enzyme SHIP2 [1]. Endocytosis, when regulated by the Eph receptor, is inhibited by the heterotypic interaction with the lipid phosphatase Ship2, via a SAM-SAM domain interaction [2]. However, the molecular interaction and regulation mechanism are not well understood. Solution NMR spectroscopy is a very versatile technique useful for the characterization of protein structure, dynamics and interactions. Here, we present the structural and dynamic aspects of the interaction of the SAM domains with the Ship2-SAM domain. We have recorded and analyzed multiple dynamic NMR experiments such as R1, R2, het-NOE relaxation, also relaxation dispersion CPMG experiments for the complexes EphA2-SAM : Ship2-SAM and EphA1-SAM : Ship2-SAM as well as the unbound proteins. The data revealed important change in the dynamics of the protein due to the complex formation, indicating key residues has changed dynamics in these interactions.

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# Functional and Biochemical Characterization of Three Recombinant Human Glucose-6-Phosphate Dehydrogenase Mutants: Zacatecas, Vanua-Lava and Viangchan

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most frequent enzymopathy in humans with a global prevalence of 4.9%. The disease is heterogeneous at genetic level with around 160 mutations described worldwide. In Mexico, 18 mutations have been described with a prevalence of 0.75%. G6PD deficiency in humans causes severe disease, varying from mostly asymptomatic individuals to patients showing neonatal jaundice, acute hemolysis episodes or chronic nonspherocytic hemolytic anemia. In order to understand the effect of the mutations in *G6PD* gene function and its relation with G6PD deficiency severity, we report the construction, cloning and expression as well as the detailed kinetic and stability characterization of three purified clinical variants of G6PD that present in the Mexican population: G6PD Zacatecas (Class I), Vanua-Lava (Class II) and Viangchan (Class II). For all the G6PD mutants, we obtained low purification yield and altered kinetic parameters compared with Wild Type (WT). Our results show that Thermal stability parameters indicate that the mutants are more susceptible to denaturation by urea and heat relative to the G6PD WT., regardless of the distance from the active site where they are located, affect the catalytic properties and structural parameters and that these changes could be associated with the clinical presentation of the deficiency. Specifically, the structural characterization of the G6PD Zacatecas mutant suggests that the R257L mutation have a strong effect on the global stability of G6PD favoring an unstable active site. Using computational analysis, we offer a molecular explanation of the effects of these mutations on the active site.

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# The mitochondrion of sea urchin sperm contains the soluble adenylyl cyclase

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Fertilization, a crucial process in sexual reproduction, requires orchestrated changes in cAMP concentrations. Speract, is a chemotactic sperm activating peptide released by the egg, that upon binding to its receptor in the flagella of *Strongylocentrotus purpuratus* sperm, triggers among other physiological changes, a signaling pathway that involves intracellular increases in pH, $Ca^{2+}$, cAMP, and NADH[1,2].
The sea urchin sperm has only one mitochondrion, which generates all the ATP the sperm needs to fertilize the egg. In this system, the main activity of adenylyl cyclase (AC; which synthesizes cAMP from ATP) is a soluble enzyme (sAC) that unlike the transmembrane ones (ACtms1-9), is regulated by $Ca^{2+}$ and bicarbonate[3,4]. Although cAMP has several effectors, its main target is the cAMP-dependent protein kinase (PKA). Both, cAMP and PKA activity are essential for sperm physiology[1]. In other systems it has been proposed that sAC and PKA are within mitochondria where they regulate oxidative phosphorylation. Indeed, the phosphorylation levels of some mitochondrial PKA substrates change when sea urchin sperm motility is activated[5]. To determine whether the sAC is within the mitochondrion of *S. purpuratus* sea urchin sperm, we isolated their mitochondria by differential centrifugation in a sucrose gradient. Antibodies were used to detect and locate sAC and PKA in isolated mitochondria performing Western blot experiments, immunofluorescence and confocal or electron microscopy. Furthermore, we measured NADH levels in sperm pre-exposed to inhibitors of sAC and PKA, and stimulated by the motility regulator speract[1,2]. Our results strongly suggest that both sAC and PKA are within the mitochondrion of sea urchin sperm where they could influence important functions of the cell such as chemotaxis and the acrosome reaction that are essential for fertilization.

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Keywords**:** Sperm, Mitochondria, Adenylyl Cyclase. Área: Bioquímica Clásica

# Phospholipases A2 isolated from centipede venoms

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Venomous animals are widely distributed in nature, their venomous glands secrete a variety of potent polypeptide toxins that act on multiple sites of preys and/or predators. These glands also produce several water-soluble enzymes that contribute to the digestion of preys and might facilitate the action of other toxic components of the venom. Some components having enzymatic activity are thought to have given raise to potent toxins. The best known example is probably that of phospholipases A2 (PLA2). These proteins occur in venom from both, vertebrate and invertebrate animals. Phospholipase A2 enzymes hydrolyze glycerophospholipids at the sn-2 position of the glycerol backbone, releasing lysophospholipids and fatty acids. They occur ubiquitously in nature as both, intracellular and extracellular forms, and hydrolyze various phospholipids. They are the most studied among all phospholipases because of their pivotal role in various biological activities and pharmacological properties. Some biochemical and pharmacological characteristics of centipede venoms have been studied. However until now the presence of enzymes showing phospholipase activity was only reported for centipedes of the species *Otostigmus pradoi*, *Cryptops iheringi, Scolopendra viridicornis, Scolopendra viridis* and *Scolopendra subspinipes dehaani*, but few information is available on its chemical structure. In this study, we reports the purification and characterization of phospholipases from centipedes *Scolopendra polymorpha* and *Scolopendra viridis* Say of the state of Morelos, Mexico. Separation of the soluble venom by high performance liquid chromatography (HPLC) permitted to obtain different fractions. The fraction eluting around 42 to 48 min showed phospholipase activity into the agar-plate containing egg yolk. Purification of this fraction permitted obtain several proteins with phospholipase activity. The enzymes were purified to homogeneity and the first amino acid residues were identified by Edman degradation. The molecular weight of the enzymes were estimated by mass spectrometry. Sequences analysis of these enzymes, showed no significant similarity to other phospholipases sequences deposited in the known public database.

# Apoptotic effects of Luteolin, Naringin and Quercetin in Head and Neck Squamous Cell Carcinoma cells

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Head and neck cancer represents the fifth most common cancer type in Mexico while globally it is ranked in sixth. It originates in the squamous cells that line the wet and mucosal surfaces of the upper aerodigestive tract including the sinuses, oral and nasal cavities, larynx and pharynx, and represents about 6% of solid tumors. It has been reported that some variants of this neoplasm reach up to 78% lethality. Moreover, in the search for alternative therapies against cancer, some studies have shown that molecules such as flavonoids may have effects that block the progression of carcinogenesis, including cellular transformation, invasion, metastasis, angiogenesis, cell cycle regulation and induction of cell death by apoptosis. Therefore, the aim of this study is to evaluate the apoptotic effects that three flavonoids, Luteolin, Naringin and Quercetin, present on the hypopharyngeal carcinoma cell line *FaDu*. For this purpose cells were treated with increasing concentrations of flavonoids and Western Blot assays were performed to assess proteins involved in apoptotic mechanisms. It was found that at a concentration of 100 µM, the three molecules inhibit Bcl-2, while they induce the expression of Bax, Bid and caspases 9 and 3. However, these effects are most noticeable with Naringin and Quercetin. In addition, DNA degradation caused by these molecules was assessed, being found that Luteolin promotes an increased degradation of genetic material. In conclusion, our data suggest that the three flavonoids have the ability to induce cell death by apoptosis in hypopharyngeal carcinoma cells.

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# Differences on the conformational substates visited by native and mutants versions of the LAOBP obtained by Accelerated Molecular Dynamics.

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Protein engineering in the realm of binding has been focused historically on mutating residues directly involved in molecular recognition. However, redesigning of binding sites has proven to be even more complex that the binding site itself. Despite efforts, the rate of success of this approach is very limited and we are still lacking for optimal design principles. Additionally, many examples show that is possible to change binding patterns by mutations outside the binding site. This fact suggest we are leaving out important details while designing, since the native structure is a dynamical ensemble.
In our laboratory, we use a periplasmic binding protein called LAO which binds positive amino acids (LLysine, Larginine and Lornithine) with nanomolar affinity as a model for understanding the interplay between dynamics and recognition. In previous works, we have designed punctual mutants for understanding the role of specific residues on its function. We took two of those: P16A and L117K. The first one shows a reduction on the rate of binding for arginine and the later shows affinity for glutamine, a ligand which the native version ignores. Both mutations are not in residues of the binding site turning the rationale of this functional difference an intriguing question. For gaining insight in the details of dynamical ensembles which were modified on these mutants we used accelerated molecular dynamics (aMD). aMD is an enhanced-sampling method implemented on amber14. This methodology allow us to compare differences on substates visited by native and mutants forms of LAOBP.

# Effect of constant IGF-I administration on insulin and its modulation by biotin

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Nutrition acts on growth through the provision of energy substrates. Food supply is the most important extrinsic factor reflected in growth. The food must be adequate both in protein and in minerals and vitamins.
Biotin is a water soluble vitamin which functions as prosthetic group carboxylase enzymes. These enzymes act on pathways of intermediary metabolism of all cell types. It described that biotin also regulates the expression of genes involved in glucose metabolism. Also, biotin supplementation induces increased release of insulin, the main hormone that regulates blood glucose levels.
Moreover, endocrine growth hormone / growth factor I insulin-like (GH / IGF-I) is the primary means used to nutrients to control the post-natal growth. IGF-I is transported in the blood bound to protein 3 specific binding to IGF-I (IGFBP-3) protein that increases stabilize addition to half-life.
In mice deficient of this vitamin has been a decrease in the gain characteristic weight and body size, not due to differences in the specific consumption of food. Furthermore, mice deficient biotin having reduced IGF-I in serum without altering levels of GH. In addition, biotin supplementation increases insulin release stimulated by glucose.
In this study the effects of administration of IGF-I on insulin and IGFBP-3 in fed diets containing different amounts of biotin mice were analyzed. The proposed hypothesis is that on the one hand, the amount of biotin is ingested in the diet directly affect the levels of IGFBP-3 and insulin. Moreover, the constant administration of IGF-I in mice deficient biotin, will cause a partial increase in body mass and a marked hypoglycemia.
MICE Male BALB / Cann three weeks of age were used with an initial weight between 11.0 and 13.0 g. They were provided deficient, sufficient food and supplemented with biotin for 7 or 8 weeks. In the fifth week of each mouse was placed a micro osmotic pump with IGF-I that constantly manages. During this time body weight, the specific consumption of food and water and serum levels of IGF-I, IGFBP-3, insulin and fasting blood glucose was measured.
The supplemented group presented similar values to those mice had enough group for all the parameters studied. Then, biotin supplementation does not alter the glycemic control, blood insulin levels, nor GH / IGF-I endocrine system. Finally, although insulin levels were decreased after administration of IGF-I in the three study groups, the difference in the amount of biotin diets did not change its response pattern.
Lower levels of IGF-I, IGFBP-3, insulin and glucose in fasting presented mice restriction biotin both before and during administration of IGF-I, while postprandial glucose was similar to sufficient group. This study main conclusion is that biotin deficiency cause a decrease in serum IGFBP-3 favoring IGF-I degradation and thus adversely affect their proliferative actions in the muscle skeletal system.

# Thioredoxin-glutathione reductase (TGR) is the main disulfide reductase expressed in the free-living platyhelminth *Dugesia dorotocephala.*

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In parasite flatworms such as tapeworms (Class Cestoda) and flukes (Class Trematoda), the redox homeostasis is dependent on a single multifunctional disulfide oxidoreductase, thioredoxin-glutathione reductase (TGR). The enzyme is able to reduce both GSSG and thioredoxin in a NADPH-dependent mode. In these organisms the typical glutathione reductase (GR) and thioredoxin reductase (TrxR), which are present in a majority of the living world, are absent. However, in 2010 the genes coding for the three enzymes TrxR, GR and TGR were found in the genome of *Schmidtea mediterranea* a free-living flatworm (Class Turbellaria). Sequences of these same enzymes in another free living flatworm (*Dugesia japonica*) were recently deposited in GenBank, suggesting the redox homeostasis between parasite and free-living flatworms could differ. In the present work we present evidence supporting the importance of TGR in the redox homeostasis of *D. dorotocephala.* Using either DTNB or GSSG as substrates to monitor TR or GR activities, the disulfide reductase activities were purified from the adult stage of *D. dorotocephala*. Electrophoretic analysis of the final preparation revealed two protein bands: a major one (over 84 % of total protein) corresponding to a protein with a molecular mass of 65 kDa, and a minor one (<16 % of total protein) of about 55 kDa. By mass-spectrometry analysis, both GR and TR were identified in the minor band, demonstrating that in *D. dorotocephala* both enzymes are expressed. On the other hand, the major protein band did correspond to TGR. Throughout selective inhibition of TGR with the gold-compound auranofin (AF), it was possible to dissect glutathione reductase activity corresponding to GR. The latter represents about 20 % of the total glutathione reductase activity in the purified fraction. Using the same strategy, the kinetic parameters were also determined. The resultant $K_m$ (136.2 µM) and $k_{cat}$ (125 $s^{-1}$) for GR were in the expected range. As regards TGR, the determined $K_m$ (11.1 µM) and $k_{cat}$ (26.5 $s^{-1}$) values with GGSG as substrate were the typical for this kind of enzyme. However, the specificity constant calculated was an order of magnitude higher when compared with the corresponding value from other TGR. In the presence of high concentrations of GSSG, TGR activity showed a hysteretic-like behavior but with shorter lag times due to the contribution of GR activity. The dependence on pH and temperature of both GR and TGR were also different. The results obtained in this work revealed that the three disulfide reductases TGR, GR, and TR are expressed as functional proteins in the free-living flatworm *D. dorotocephala.* Nonetheless, TGR still represents the main disulfide oxidoreductase, as in its parasite counterparts.

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# Phosphorylation Sites in the Free Fatty Acids Receptor 1, FFA1 (GPR40)

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The GPCR superfamily contains around 800 members in the human genome. These proteins are very important in physiology but also are therapeutic targets for the treatment of many diseases. The fatty acid receptor family (GPR40, 41, 43 and 120) is an example of GPCRs that have been recently investigated; until a few years ago, their endogenous receptor ligands and signaling pathways were unknown.

Specifically FFA1 (GPR40), a member of fatty acids receptor family, is a protein of 300 amino acids, mainly expressed in pancreatic β cells and enteroendocrine cells. In pancreas, activation of FFA1 induces glucose-dependent insulin release; and in the gut, this receptor promotes incretin release.

FFA1 activation occurs in the presence of long chain fatty acids such as docohexanoic acid (DHA) and linolenic acid (LA); activation produces an increase in intracellular calcium, suggesting that this receptor is coupled to the Gq/phospholipase C/ $InsP_3$-DAG pathway. Its activation also stimulates the MAP kinase pathway.

Previous studies in our laboratory showed that FFA1 is a phosphoprotein whose phosphorylation state is increased by the presence of long chain fatty acids such as DHA and linolenic acid α (α-LA); after receptor activation, FFA1 is internalized. Both DHA and α-LA increase intracellular calcium concentration and promote the phosphorylation of ERK1/2, a member of the MAP kinase family. In addition, it was also shown that PKC activation is iinvolved in receptor phosphorylation. The aim of our study is to determine the specific phosphorylation sites in the intracellular portions of FFA1, as an initial step to get insights on the functional consequences of these posttranslational modifications.

To achieve our goal, we generate a cell line from HEK 293 cells, expressing the FFA1 receptor fused to the GFP (green fluorescent protein) in an inducible expression system (T-Rex system). Subsequently, we standardize the purification protocol of FFA1-GFP by immunoprecipitation using a commercial resin with antibodies against GFP. In the first approach we used three conditions: basal, presence of DHA (30μM, agonist) or PMA (1μM, PKC activator). Immunoprecipitation products were separated on SDS-PAGE (10%); gels were stained with Coomasie blue to make visible the protein bands. We cut the bands corresponding to FFA1-GFP (58kDa), identified by Western blotting, and were sent to mass spectrometry analysis. The initial site identification will be presented in the meeting. In silico analysis showed the following potential phosphorylation sites: threonine-39 in the first intracellular loop; threonine-215 in the third intracellular loop; threonines-287 and -293, and serine-298 in the carboxyl terminus.

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# The role of three-pyruvate kinases present in *Vibirio cholerae*.

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In contrast to other bacteria, *Vibrio cholerae* (a gamma-proteobacterium) has three pyruvate kinase: VCIPK, VCIIPK and VCIIIPK. Its genome consists of two circular chromosomes; VCIPK and VCIIPK are located in chromosome 1 and VCIIIPK in chromosome 2 (megaplasmid). In general this megaplasmid contains duplication of genes. The individual roles of VCIPK and VCIIPK were previously studied (not published). VCIPK and VCIIPK were $K^+$-dependent and $K^+$-independent enzymes, respectively. This result is coincidental with a previous phylogenetic study of the family of piruvate kinase, where it stands that those sequences that have Glu117 are $K^+$-dependent, whereas those with Lys117 are $K^+$-independent enzymes (Oria-Hernández, J. *et al.* (2006) *J. Biol. Chem.* 281, 30717-30724). In this respect, VCIIIPK should be a $K^+$-independent enzyme. The goal of this study is to understand why *V. cholerae* possesses three isoenzymes, and why does it have two $K^+$-independent pyruvate kinases?

In this work we studied VCIIIPK. Initial trials to produce VCIIIPK/pET-$His_6$Tag in *E. coli* BL21DE3-Codon Plus strain resulted in the production of large amounts of recombinant protein in insoluble form, indicating that this enzyme was mainly produced in inclusion bodies. The yield was 2.7 mg/L. This behavior was different in comparison with the other two isoenzymes. As expected, VCIIIPK was $K^+$-independent and preliminary results indicate that VCIIIPK is not activated by either fructose 1,6 bisphosphate or ribose 5 phosphate as VCIPK and VCIIPK are, respectively. In this respect, it is relevant to mention that VCIPK is a constitutive enzyme whereas VCIIPK is an inducible one. This research was supported by DGAPA-UNAM IA204816.

# Study of the mechanisms underlying enzyme release from hepatic and lung tissues to the extracellular milieu in rats subjected to 70% partial hepatectomy.

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The liver has the outstanding capacity of regenerating as a response to an important loss of hepatic mass. This process which is strictly regulated, allows the maintenance of the balance between the number of cells and the content of components constituting the extracellular matrix, which acts as a support for the liver cells. During the restitution of the liver mass after surgery, driven by active cell proliferation of liver cells (hyperplasia), there are characteristic patterns of enzyme release into the bloodstream, which seems to be also a controlled event. Despite of the mechanisms controlling the rate of enzyme release are not well defined, we have demonstrated that this phenomenon can depend on at least two events: 1) The action of blood flow-bearing physical forces (mechanotransduction), and 2) Mechanisms that are independent of changes in the hemodynamic forces [1]. The present work was addressed at investigating the possible mechanisms involved in the flow-independent control of enzyme release from either, liver and lung; for this, we used in an *in vivo* model represented by male Wistar rats undergoing two-thirds partial hepatectomy (PH). We selected specifically the release of the following enzymes: Lactic (LDH) and glutamic acid (GDH) dehydrogenases, alanine (ALT) and aspartic acid (AST) aminotransferases, as well as the recognized more specific hepatic enzymes, namely ornithine carbamoyl-transferase (OCT) and arginase. The selection of this set of enzymes was based upon the so-named enzymatic “maps” or “models” for a specific tissue. Each liver enzyme tested showed a unique or particular kinetics of release in the control animals. In liver samples obtained from rats subjected to PH, there was a selective increase of enzyme release at 24 hr after the surgery, time when DNA synthesis is maximally increased [1]. Interestingly, when liver samples were taken 48 hr after PH (after the peak of mitosis), the profile of hepatic enzymes was quite similar to that in controls. Results therefore indicate that increased enzyme release from the proliferating liver is an early event during progression of rat liver regeneration. Since the liver is an important element interconnected with the rest of the body organs and tissues, it is likely to suppose that changes in the hepatic metabolism and homeostasis, such as occurs after a PH, can affect other organs. Therefore, we also looked for the possibility that lung enzymes can be also released into the extracellular milieu. In this organ, we could also observe a characteristic release of some enzymes, but not all. In fact, we detected an increased amount of OCT in the lung of rats subjected a PH, only after the first day of surgery. Then, it is possible that after PH, specific liver enzymes released into the bloodstream can reach and to be incorporated into other organs, as the lung. In conclusion, we think that an important fraction of the enzymes released by the liver are transported as a “particulate” form, probably in the currently known exosomes, which can prevent degradation and allow recognition by other cells. Therefore, we are now testing this hypothesis searching for different physics-chemical states of the released enzymes.

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# Recovery of abundant chondrocytes after differentiation of sheep bone marrow CD90+-mesenchymal stem cells mobilized to peripheral blood and expanded in culture medium with defined fetal bovine serum.

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**Introduction.** Due to the limited regenerative capacity of cartilage, its repair requires the implantation of chondrocytes densely adhered to biocompatible scaffolds. The recovery of chondrocytes from human cartilage biopsies in sufficient quantity forces their expansion by repeated passages that ultimately result in their dedifferentiation. Here we explore the alternative in sheep to first expand great quantities of bone marrow (BM) CD90+-mesenchymal stem cells (CD90+-MSC) mobilized to peripheral blood (PB) and then differentiate them to abundant chondrocytes, characterized by their morphology and gene expression profile, without the need of additional passages that would lead to their dedifferentiation. **Objective.** To obtain abundant chondrocytes by differentiation of great quantities of BM CD90+-MSC mobilized to PB of a ewe after administration of granulocyte colony stimulating factor (GCSF) and *in vitro* expansion in DMEM culture media supplemented with adult sheep serum (ASS) or defined fetal bovine serum (dFBS). **Methods.** After a quarantine period, a Suffolk ewe weighing 82 kg, with a normal complete blood count, was given sc GCSF (10 µg/kg/24h) for 3 days to mobilize MSC from BM to PB. At the end of the treatment, 20 mL of blood were collected via the jugular vein. Blood mononuclear cells (16 x $10^6$) were obtained by Ficoll gradient. From them, CD90+-MSC (8.2 x $10^6$) were isolated using superparamagnetic beads coated with anti-CD90+ antibody. Using flow cytometry, their immunophenotype was defined by CD73+/CD105+/CD90+, and CD14-/CD34-/CD45-. These cells were expanded in DMEM with 10% ASS or 20% dFBS, and then differentiated into chondrocytes in condrogenic DMEM (conditioned medium or in co-culture with chondrocytes, with or without TGFβ1 or BMP7) with 10% ASS. Their state of differentiation was verified by morphology and their gene expression profile of GADPH, AGR, COMP, COL I and II, SOX9, RUNX2, OCN, OPN and COL X by end-point RT-PCR. This study was performed in compliance with current legislation for experimentation in animals. **Results.** BM CD90+-MSC mobilized to PB and expanded in DMEM with ASS, showed a spherical morphology, which did not change in response to our attempts to differentiate them into chondrocytes in chondrogenic culture media or in co-culture with chondrocytes, with ASS, with or without TGFβ1 or BMP7; besides, under any experimental condition, their gene expression profile was always the same: GADPH and AGR. Conversely, the same CD90+-MSC expanded in a culture medium with dFBS, resulted in their abundant proliferation now with a fibroblastic phenotype, that we were able to differentiate in a chondrogenic culture medium, with ASS and BMP7, into a cellular type with a morphology and similar gene expression profile (in similar proportions) to that of primary chondrocytes from femoral sheep cartilage, namely, GADPH, AGR, COMP, COL I and II, SOX9, OCN, OPN y COL X; neither of them expressed RUNX2. **Conclusions.** Although similar to the gene expression profile of primary chondrocytes from femoral sheep cartilage, that of the differentiated BM CD90+-MSC mobilized to PB showed a state of hypertrophic differentiation. Once implanted into injured cartilage, it will be necessary to determine if they retain their phenotype or become ossified.

# Studies of Molecular Docking of Nicardipine and Nitrendipine on Aldose Reductase

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Aldose reductase enzyme (AR) catalyses the first reaction of the polyol pathway (the irreversible reduction of glucose to sorbitol). In hyperglycemia conditions there is an overactivation of this pathway with consequent sorbitol overproduction, which not rapidly transformed to fructose (by sorbitol dehydrogenase enzyme) nor disseminated freely through membranes. It is contributing to increase intracellular osmotic pressure, resulting in tisular damage due to cellular edema. AR has been identified as an important factor in the genesis and development of cataracts, as well as of neuropathies and vascular damage that occur in patients with chronic diabetes. Based on, it has been proposed that AR inhibition could contribute to reduce or delay the onset of secondary complications of diabetes. Recently, we have found that several drugs of type 1,4-dihydropyridine (DHP) can inhibit the AR. DHPs are included in the group of drugs known as calcium channel blockers (CCBs) and several DHPs are used therapeutically in the treatment of hypertension and other cardiovascular diseases. Although it has been reported that DHPs may have other biological actions independent of its effect as BCC (like antitrypanosomal effect, antileishmanial activity, etc.). In order to determine the DHPs binding site in AR, molecular docking studies were performed for two DHPs: nicardipine (Nic) and nitrendipine (Nit). For these studies the AutoDock Vina software (v. 1.1.2) and the Maestro program (v. 9.8, Schrodinger Inc.) were used. Ligands molecules (Nic, Nit and fidarestat, an AR competitive inhibitor) were generated in the Maestro program, in which likewise were optimized the chemical structures (by the semiempirical method AM1), while the three-dimensional structure of AR (AKR1B1) was obtained from Protein Data Bank page. The results show that both Nic and Nit, like the fidarestat, bind to the catalytic site of the enzyme, suggesting that the DHPs are competitive inhibitors of AR.

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# Dehydrin profile comparation in three maize landraces under drought stress

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## Abstract

Dehydrins (DHNs) or group 2 LEA (Late embryogenesis abundant) proteins, also referred as RAB (responsive to ABA), play an important role in the response to abiotic stresses such as dehydration, salinity, cold and freezing. LEA proteins have been found in plants, bacteria and some invertebrates. DHNs are highly hydrophilic proteins characterized by the presence of the K-segment, a highly conserved motif (EKKGIMDKIKEKLPG). It has been suggested that the interaction between this motif and the cellular membranes stabilizes them under stress conditions.

In this study, we compared the DHN profile in three different maize landraces as possible indicators of drought stress: L.14 and L.13 developed by Colegio de Postgraduados through conventional breeding; L.14 is more tolerant than L.13. A third one (Tuxpeño) considered as a drought-tolerant wild type landrace was also analyzed. Maize plants from the three landraces were maintained one week under drought stress. Then seeds, roots and leaves were analyzed. The survival rate and the relative water content measured in maize plants showed that L.14 was more drought-tolerant than the other two. We observed that L.14 plants had better drought stress survival and lower water loss during the treatment. The western blot analysis showed the presence of the three dehydrins with different molecular weight in the L.14 and Tuxpeño leaves, and only two in the L.13 leaves. The 25 kDa DHN was more abundant in the L.14 than in the other two landraces. DHNs there were not found in the roots of the three lines studied. The DHN found by inmunodetección was identified by mass spectrometry as RAB17 or DHN1. In maize seeds we found two different DHNs and again the more abundant was in the L.14 landrace. These results strongly suggest that RAB17 participates in maize drought-tolerance and might be a useful characteristic in maize plants.

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# Residual structure of a motif in the intrinsically disordered region of the Escargot protein.

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Many proteins adopt a unique three-dimensional structure determined by their amino acid sequence. However, around of 30% of the eukaryotic proteins are disordered and least 70% have disordered regions, known as intrinsically disordered proteins (IDPs) or intrinsically disordered regions (IDRs) respectively. The IDPs and IDRs lack stable structure, adopt a variety of conformations, are highly flexible, and have regions known as MoRFs (Molecular Recognition Features), which are essential for binding to their targets[1]. Proteins such as transcription factors are enriched in IDRs. The Snail family is comprised of transcription factors that bind to DNA, RNA, and proteins, and regulates multiple cellular functions. There are four Snail genes in *Drosophila melanogaster* (Snail, Escargot, Worniu and Scratch). Escargot (Esg) presents five zinc-fingers in the C-terminal domain, which are conserved in the family and necessary for transcriptional regulation and binding to DNA. On the other hand, the N-terminal domain is divergent, and the only functional annotation in this region consists of two motifs (P-DLS-K) that interact with the C-terminal binding protein (CtBP) [2,3]. In this project we identify, through molecular dynamics (MD) simulations, the presence of MoRFs in the intrinsically disordered region of N-terminal domain of Esg and its homologs. The disorder profile of this region revealed a ~50 amino acid motif that is partially ordered, and its local structure, characterized by an alpha helix and a few long-range contacts, persists during MD simulations. The residual structure at this region may be involved in the regulation of Esg activity, through interactions with other proteins.

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# Nitric assay evauation in several samples of plasma and serum

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Abstract

The nitric oxide (NO) plays an important paper in the biological systems, acts like second messenger, neuromodulation and molecular effects. An effective method for the quantitation of NO is mediated the quantitation of nitrites ($NO^-_2$) that was described by Griess and modified over the years. One of the problems to make the Griess reaction is the interference of many substances like proteins, lipids or the matrix where is developing the reaction. The nitrites have a stable intermediary that is not reduce completely: the nitrates, so that in necessary reduce with a nitrate reductase enzyme or well with granular cadmium for detect the nitric oxide total (nitrates plus nitrites). The aim of this work is compare the concentration of nitrites in serum and plasma, its dependence with the quantity of reagent of Griess, if exist interference of proteins, lipids and/or hemolysis, in treated samples with or without cadmium and if interfered the time of storage in which maintain the samples at 4°C. Was they recruited 10 patients for the obtaining of the sample, that was obtaining for venipuncture in the different kinds of tubes, it was centrifuged and it was aliquoted the serums or plasmas for the next analyses. The determination of nitrites it was realized with the Griess technique and the Griess technique modified with granular cadmium. For serum or plasma determinations were made which one with two variables, a samples with treatment using deproteinizing agent to zinc sulfate 30% and another samples without treatment. To ensure accurate quantification it was made for each assay a reference curve with the nitrite standard. It was analyzed like matrices to distillated water, demineralized water, water for electrophoresis, and buffer of phosphates. The results show that independently of the kind of water, either distillated, demineralized, or for electrophoresis, don't exist some difference, but nevertheless when is utilized PBS although it was show an increment with the buffer, this one isn't statistically signified with respect to the curve with water, the samples for its part show a dependence to the quantity of the protein present including to the hemoglobin, so that included an extra step in the modified technique of Griess that was the centrifugation, after to this process it was observed a diminution in the interference, finally reduction with cadmium was favored in ambient temperature for thirty minutes.

# Study of osmotic fragility of erythrocytes in Chemistry students UJAT

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## Sumary

The osmotic fragility test is a measure of the resistance of erythrocytes to hemolysis osmotic stress, which depends mainly on the volume of the cell, its surface area and its membrane function. The present study was carried out an analysis of the osmotic fragility of erythrocytes in blood samples from 25 healthy students of the degree in Chemistry in the UJAT university, which were randomly selected, of which 7 were men and 18 women between 21 and 24 years old. Heparinized samples were subjected to a centrifugation process using a Hellix 32A equipment, for a period of 10 min at 2500 rpm, eliminating the plasma substance and washing triplicate cell package with a buffer solution phosphate at pH 7 they were prepared 14 dilution test tubes at different concentrations of NaCl 0.5%, to which was added 0.05 mL of the erythrocyte suspension 25% phosphate buffer, same that were left to rest at room temperature (25oC) for 30 minutes and finally centrifuged. Subsequently conducted to a visual and spectrophotometric analysis at 540 nm. Results from visual analysis, denoted forming a red button suspended in the clear supernatant, indicating the presence of cell lysis in the tubes 10, 12 and 14, wherein the osmotic fragility of erythrocytes is high. The minimum globular resistance was observed in solutions with 0.45% NaCl and the maximum globular resistance occurred from 0.35% NaCl. For minimum globular resistance was taken as reference value 10% hemolysis, which is representing a maximum allowable value to consider hemolysis within normal limits and was taken as reference value for the maximum globular resistance 90% hemolysis. Based on the results it was concluded that the ability of normal erythrocytes to resist tonicity hypo comes from its biconcave shape and elastic properties of the membranes, which allow the cell to increase its volume up to 70% before the membrane is stretched; once the hemolysis limit is produced.

# MicroScale Thermophoresis for the study of (Bio)molecular Interactions

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We present MicroScale Thermophoresis (MST), an immobilization-free technology for quantitating intermolecular interactions, ranging from ion-protein to protein-protein interactions. MST, the directed movement of molecules in optically generated microscopic temperature gradients, is monitored by fluorescence. This thermophoretic movement is affected by the entropy of the hydration shell around molecules and is highly sensitive to binding and oligomerization reactions, which affect the size, charge, conformation, and/or hydration. Measurements are not limited to specific buffer conditions and can be performed with vesicles/liposomes, in cell lysate or blood serum. We show how MST can be used to probe stoichiometry, thermodynamics, and binding mode. In addition, we demonstrate how interactions of proteins can be quantified in a label-free manner using intrinsic tryptophan fluorescence.

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# "The effect of punctual mutations on the stability and aggregation state of human CGI-58 protein "

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Protein X-ray crystallography is a methodology that allows knowing the protein three-dimensional structure. However, growing good diffracting crystals have shown a strong dependence on protein characteristics like solubility and stability. Because of that, obtaining crystals of several proteins have shown to be a challenge, that is the case of CGI-58, a protein involved in lipolysis. Lipolysis occurs in white adipose tissue during times of energy demand, where the cells hydrolyze the triglycerides to fatty acids (FA) and glycerol as energy substrates using three lipases: ATGL, HSL and MGL. CGI-58 activates ATGL, the first lipase from lipolysis, increasing its activity and extending the selectivity of FA hydrolysis from sn-2 to sn-1 position. In the literature, it has been reported that some mutations in CGI-58 prevent it from activating ATGL and causing the Chanarin-Dorfman syndrome in humans, which is a very rare neutral lipid metabolism disorder with multisystem involvement. In our laboratory, we are interested in determining the crystal structure of ATGL and CGI-58; those are key to understand the mechanism by which CGI-58 activates ATGL. However, these proteins present solubility and stability problems. In the case of CGI-58, we have made point mutations in amino acids that are involved in its binding to the lipid droplet or that are involved in phosphorylation sites. These mutations have enabled us to increase protein solubility, improving purification yields. Moreover, by the Thermofluor technique, we have determined that the point mutations in CGI-58 increase its thermal stability up to 15 ° C with respect to the wild type protein. In conclusion, we have a version of the CGI-58 protein that is more soluble and stable than the wild type allowing us to perform crystallization assays.

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# Energetic bases of multidomain functioning of $F_1$-ATPase β subunit.

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The $F_0F_1$ ATP synthase has been divided into two sectors: on the one hand the hydrophobic sector ($F_0$ $ab_2c_{9-12}$), which is embedded in the membrane, and secondly, the hydrophilic sector ($F_1$ $\alpha_3\beta_3\gamma\delta\varepsilon$) projecting solvent. $F_1$ is also known as $F_1$-ATPase, because has the ability to hydrolyze ATP in the absence of F0. Both sectors are joined by a central stalk and a peripheral stalk.

Within the structure of $F_1$, the catalytic core is formed by a cylinder hexameric of 3 subunits α and 3 subunits β (catalytic) interleaved and with the γ subunit in the inside of the hexamer. The catalytic sites are located at the interfaces of α / β.

Conformational changes induced by nucleotide binding to the catalytic subunit β play a crucial role in the mechanism of rotation $F_1$ sector of ATP synthase.

In order to gain knowledge about the energetic bases governing recognition nucleotide by the isolated β subunit from thermophilic bacteria *Bacillus PS3*, a characterization of the binding of this monomer adenosine nucleotides was performed using isothermal titration calorimetry of high precision to determine whether the union is coupled to an exchange of estructurals waters, protons and / or counterions.

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# Characterization of cyclin proteins in *Trichomonas vaginalis*

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Most cells undergo an orderly sequence of coordinated events to divide themselves and to generate two new genetically identical cells, a process known as cell cycle. For its study, the cell cycle is divided into four phases: G1, S, G2 and M phase.
During the cell cycle, is required the activity of different molecules, which are responsible of regulation and control the cell cycle progression. The components of this control system, are the members of a family of kinase proteins known as cyclin-dependent kinases (Cdks) that do not display their kinase activity, unless they associate with a cyclin protein.
Cyclins are characterized by the oscillation of their concentration levels which are dependent on synthesis and degradation throughout the cell cycle. Based on their behavior during the cell cycle, these proteins can be classified into four types as cyclins D, E, A and B. Generally, amino acid sequences of the cyclins are very different from each other, however, the homology between them is limited to a relatively conserved domain of about 100 residues termed cyclin box, which comprises the binding site to the Cdk.
Regulatory cell cycle proteins have been extensively studied in yeasts, plants and higher eukaryotes, however, these important regulators in protozoa, are few characterized.
*Trichomonas vaginalis* is a microaerophilic protozoan of early evolutionary divergence that cause trichomoniasis, a non-viral genitourinary transmitted disease in humans. *T. vaginalis* is asexually reproduced and divides by binary fission. Studies on cell cycle of this protozoan have not been described yet, however, the steps comprising mitosis have been well characterized by electron microscopy.
Furthermore, there are no studies about cyclins in *T. vaginalis*. By BLAST analyses, at least 26 putative cyclins have been identified in the *T. vaginalis* genome. All of them contain the cyclin box and other characteristic motifs of cyclin proteins. In our relevant study, four sequences were selected as potential cyclin proteins based on their motifs.
By complementation assays with a *Saccharomyces cerevisiae* cyclin knockout, it was determined that three of these putative proteins rescues the yeast cyclin function, therefore suggesting that *T. vaginalis* putative cyclins may be involved in cell cycle regulation. Additionally, with pull-down assays, interaction with *T. vaginalis* Cdks was analysed, which strongly suggests their function as cyclin proteins.

Finally, phosphorylation assays using a histone H1 as substrate were performed. They determined that at least three of the studied cyclins are able to activate a *T. vaginalis* Cdk to induce its cyclin-dependent kinase activity.
Altogether these results indicate that there are some cyclin molecules in *T. vaginalis* that share conserved features seen at sequence and at protein levels that need to be further characterized. Our studies suggest that these molecules may have an important role as cell cycle regulators in *Trichomonas vaginalis*.

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# Analyzing protein-ligand interactions using ancestral protein reconstruction.

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A key goal of biochemistry is to determine the function and the physicochemical properties of a desired protein sequence. To address this question, several aspects need to be determined i.e: How the sequence of a protein determines its three-dimensional structure? How the proteins fold so quickly and specifically? What is the basis of their physicochemical properties, such as its specificity and activity? (Harms et al., 2013). Phylogenetic analysis of protein sequence data to generate ancestral proteins could be an strategy to determine the regions or residues that confer new binding and catalysis properties in a family of homologous proteins.

The ancestral protein reconstruction has been used to study the evolutionary history of protein function. This can provide additional information about promiscuous protein functions. This information is difficult to obtain with the extant proteins that are limited to the current range of studied available functions. In addition, providing the information about the evolution of extant proteins, can lead to the discovery of new aspects of biochemical functions that have been lost or gained in proteins (Chang et al., 2005).

This work proposes the reconstruction of the evolutionary history of binding proteins, which are an example of how nature has changed the binding specificity to different ligands and retained their overall structure (despite of the sequence diversity). This analysis will provide information about changes in the sequences that led to modify the affinity/specificity for their ligands and will further grant clues to understand the combinatorial events that led to this diversity. Further, the ancestral protein reconstruction will be applied to redesign protein-ligand interactions.

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# Easy and Rapid Analysis of Protein Stability by nanoDSF

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nanoDSF is an advanced Differential Scanning Fluorimetry technology. It detects smallest changes in the fluorescence of tryptophan present in virtually all proteins.
The fluorescence of tryptophans in a protein is strongly dependent on its close surroundings. By following changes in fluorescence, chemical and thermal stability can be assessed in a truly label-free fashion. The dual-UV technology by NanoTemper allows for rapid fluorescence detection, providing an unmatched scanning speed and data point density. This yields an ultra-high resolution unfolding curves which allow for detection of even minute unfolding signals. Furthermore, since no secondary reporter fluorophores are required as in conventional DSF, protein solutions can be analyzed independent of buffer compositions, and over a concentration range of 250 mg/ml down to 5 µg/ml. Therefore, nanoDSF is the method of choice for easy, rapid and accurate analysis of protein folding and stability, with applications in membrane protein research, protein engineering, formulation development and quality control.

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# Cloning and Expression of ribosomal GTPase LSG1

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SUMMARY

GTPases are regulatory enzymes involved in essential cellular processes such as signal transduction, cytoskeleton formation and ribosomal biogenesis (Karbstein, 2007).

In *Saccharomyces cerevisiae*, ribosome biogenesis requires the coordinated action of 75 small nucleolar ribonucleoproteins (snoRNP), and 200 accessory proteins that are not present in translationally competent ribosome (Henras, et al, 2008). In very few cases it is known in detail the role of these accessory proteins. Lsg1 is a GTPase involved in the final maturation step of the 60S subunit that occurs in the cytoplasm. Lsg1, along with the protein Rpl10 are involved in the release of the Nmd3, an export adapter for the pre-60S subunit through the nuclear pore. In turn, Rpl10 requires interaction with the chaperone Sqt1 to join the 60S ribosomal subunit. Rpl10 binding is necessary for the release of Nmd3, either because it provides a binding site for Lsg1, or because it affects the activity of GTP hydrolysis Lsg1 (Karbstein, 2007).

The aim of this work was to produce recombinant GTPase Lsg1. We tested the expression in two different systems, yeast and *E. coli*. The protein was successfully expressed in bacteria and a purification method established with a yield of 3.5 mg/L of culture. The folding of the protein was studied by circular dichroism, dynamic light scattering and fluorescence spectroscopy. Preliminary characterization of its binding activity was obtained by fluorescence with a Kd for the non-hydrolysable substrate analogue GppNHp of 150 uM .

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# Paralogous Diversification: Repercussion on Metabolic Fluxes

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The genome of the yeast Saccharomyces cerevisiae contains duplicated regions, originated from a Whole Genome Duplication (WGD) event. Duplicated gene retention and diversification could have provided the metabolic and regulatory mechanisms, which participate in the global rewiring needed to acquire facultative metabolism: namely, channeling the use of intermediates to either energy or biomass providing pathways. However, we lack mechanistic examples of such adaptive role of gene duplication in metabolism. These project pretends to: (a) Analyze functional diversification of selected WGD-paralogous genes involved in amino acid biosynthesis in S. cerevisiae compared with the functionality of their pre-duplication orthologues in the aerobic yeasts *Kluyveromyces lactis* and *Lachancea kluyveri*, and (b) develop a metabolic and transcriptional dynamic model linking central carbon and amino acid metabolism to study the role of paralogous functional diversification on the metabolic switch between respiration and fermentation, thus identifying the mechanisms leading the adaptation to facultative metabolism.

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# Orai1 channel modulates agonist-induced $Ca^{2+}$ release from the endoplasmic reticulum.

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The calcium ($Ca^{2+}$) ion as a second messenger participates in the regulation of different cellular processes such as hormone secretion, muscle contraction, gene transcription and apoptosis (1). These processes are induced by elevations in the cytoplasmic calcium concentration ($[Ca^{2+}]_i$) where the two main sources are the extracellular medium and the endoplasmic reticulum (ER).

It has been described a $Ca^{2+}$ entry mechanism denominated SOCE (Store operated-calcium entry); this is a common and ubiquitous mechanism of $Ca^{2+}$ influx (2). There are two different families of proteins involved in this pathway: Orai channels and luminal $Ca^{2+}$ sensor STIM, they reside at the plasma membrane and endoplasmic reticulum, respectively. Orai1 E106A mutant demonstrated that this aa is essential for the high $Ca^{2+}$ selectivity while STIM has a single EF-hand in the intraluminal side of the ER that allows oligomerization of these proteins when $Ca^{2+}$ level has been reduced; this in turn results in STIM interaction with Orai1 channels promoting $Ca^{2+}$ influx from the extracellular medium to cytoplasm and refilling of the ER $Ca^{2+}$ store by the action of SERCA pump (3).

On the other hand, it has been shown that PKC regulates SOCE by phosphorylating Orai1 in S27/S30 residues, causing a reduction in $Ca^{2+}$ entry. These results suggest that $Ca^{2+}$ influx can be downregulated by PKC (4).

Furthermore, it has been observed that Orai1 interaction with $IP_3R$ is enhanced by agonists that activate PKC (5). Data from our laboratory have shown that Orai1 overexpression reduces the agonist-induced ER $Ca^{2+}$ depletion as a late effect, suggesting that Orai1 channel might also have a regulatory role in $Ca^{2+}$ releasing mechanisms.

To study this new role of Orai1 channel, we performed simultaneous measurements of $[Ca^{2+}]_i$ (Fura-2) and luminal ER calcium changes (Mag-Fluo-4) and we have observed that overexpression of Orai1 stimulated the initial agonist-induced $[Ca^{2+}]_i$ response. This effect required a functional Orai1 channel. Later on, it appears that Orai1 switches from SOCE to reducing activity of $IP_3Rs$ an action that does not require a functional channel but PKC-mediated phosphorylation of S27/S30 residues. Accordingly, Gö 6976, a specific inhibitor of classical PKC, inhibited the late effect of Orai1 on ATP-induced ER depletion. Collectively, these data suggest a scenario where Orai1 facilitates refilling of the ER $Ca^{2+}$ store by a dual mechanism as a $Ca^{2+}$ permeable channel and as a direct inhibitor of $IP_3R$ activity.

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# $S1P_1$ receptor differentially associate with Rab proteins upon sphingosine 1 phosphate and FTY720-P.

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$S1P_1$ receptor is seven transmembrane-spanning protein that mediate the actions of sphingosine 1-phosphate (S1P), a bioactive sphingolipid metabolite that mediates a plethora of actions at the levels of individual cells, tissues and organisms. This lipid modulates proliferation, promotes survival, and vesicular trafficking among many other cellular effects. In contrast, is involved in pathological processes such as fibrosis, cancer and multiple sclerosis (MS). $S1P_1$ receptor is expressed on the surfaces of lymphocytes and is important in regulating egression from lymph nodes. MS is a chronic autoimmune and neurodegenerative disease of the central nervous system (CNS) associated with irreversible progression of disability. Fingolimod (FTY720-P) initially activates lymphocyte $S1P_1$ via high-affinity receptor binding, yet subsequently induces $S1P_1$ down-regulation that prevents lymphocyte egress from lymphoid tissues, thereby reducing autoaggressive lymphocyte infiltration into the CNS.
Internalization of G protein-coupled receptors can be triggered by agonists or by other stimuli.The Rab GTPase family controls endocytosis, vesicular trafficking, and endosomal fusion. In the endocytic pathway, Rab 5 controls traffic from the plasma membrane to early endosomes. Moreover, Rab 7 and Rab 9 regulate the traffic from late endosomes to lysosomes and recycling to the trans-Golgi.

It is known that S1P and FTY720-P differentially phosphorylate $S1P_1$. Nevertheless, studies focused on the receptor vesicular traffic elicited by S1P and FTY720-P are still limited. Therefore, the objective of this work was to characterize the kinetic phosphorylation and vesicular traffic involved in $S1P_1$ receptor. In order to achieve this, we used a combination of intracellular calcium determinations, $S1P_1$ receptor phosphorylation and vesicular traffic. We found S1P is more potent than FTY720-P when measuring intracellular calcium concentration. Additionally, we found a differential receptor phosphorylation kinetic upon S1P activation as compared to FTY720-P. Furthermore it was observed that, when $S1P_1$ receptor was stimulated with S1P, the receptor interacted with proteins present in early endosomes, such Rab5 and with late endosome markers, such as Rab 9, but not Rab7. Upon FTY720-P, the receptor interacted with Rab5 and with late endosome markers, such as Rab 7, but not Rab9.

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# Physicochemical characterization of Triosephosphate Isomerase in the phylum Proteobacteria.

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Triosephosphate Isomerase (TIM) is one of the most important glycolytic enzymes, it is recognized as a perfect and evolved enzyme due to it´s high catalytic efficiency, limited only by substrate diffusion to the active site. The structure, activity and folding of TIM from eukaryotes has been thoroughly studied. Although less is known about bacterial TIM´s; in recent study (Romero-Romero, *et al*. 2015), we found that three bacterial TIM´s show reversible thermal unfolding.

Within bacteria, the proteobacteria phylum is the most diverse, not only in the number of species, but also in their metabolism, habitat, and lifestyles. The TIMs form this phylum provide thus the opportunity to explore how this biochemical diversity is related to physicochemical properties and folding pathways of enzymes that are closely related. The TIMs selected for experimental characterization where chosen by sequence alignments, and some physicochemical properties that could be related to the reversibility of this enzymes. The selected species where: *Brucella melitensis*, *Burkholderia thailandensis* and *Geobacter daltonii* an alpha, beta and epsilon proteobacteria respectively.

Results on their activity; secondary and quaternary structure; thermal and chemical unfolding will be presented. This study is supported by PAPIIT IN220516, CONACYT 254514 and Facultad de Medicina UNAM.

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# The amoebic oxygen reduction pathway is constitutive and increases under *in vitro* hyperoxia and during liver abscess formation in hamsters

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Both *Entamoeba histolytica* and *Entamoeba dispar* display an oxygen reduction pathway (ORP) composed mainly by flavodiiron protein ($O_2 \rightarrow H_2O$), Eh34 protein ($O_2 \rightarrow H_2O_2$) and peroxiredoxin ($H_2O_2 \rightarrow H_2O$). The ORP is important to resist the low $PO_2$ concentration of 0.2-2% present in the human large intestine; however, the higher ORP protein content in *E. histolytica* may enable it to resist both the higher tissue $PO_2$ (4-16%) and the $H_2O_2$ derived from the host response during tissue invasion. Given the relevance of the ORP to resist oxidative stress, in this work the protein profile of the ORP was determined under *in vitro* oxidative conditions ($O_2$ and $H_2O_2$), and during the development of amoebic liver abscess in hamsters (ALAH). ORP proteins were detected by confocal microscopy using polyclonal specific antibodies obtained from rabbits after immunization with recombinant proteins. In addition, oxidative tissue status was determined by *in vivo* reduction of nitroblue tetrazolium to formazan. Our results showed increased expression of the three ORP proteins under *in vitro* oxidative stress, with enrichment in the lateral surface of trophozoites. However, ORP proteins were increased in ALAH only during the late stages of infection, which correlated with high tissue oxidative stress. Taken together, amoebic ORP protein expression is constitutive and its overexpression during the exposure to oxidants is expected to be regulated at posttranscriptional level. Experiments to demonstrate the relevance of ORP for the parasite pathogenesis are being carried out in our lab.

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# B-dystroglycan is involved in the nuclear envelope organization of differentiated HL-60 cells

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## Abstract

B-dystroglycan is an integral membrane protein of 43 kDa encoded by the gene DAG1 in human. Due to its broad expression pattern and its interaction with multiple proteins, the β-dystroglycan is considered a multifunctional protein involved in adhesion, basement membrane assembly, signal transduction, reorganization of cytoskeleton, formation of neuromuscular junctions, cancer and nuclear envelope organization.

Recently has been characterized the presence of β-dystroglycan in different blood tissue cells as part of the dystrophin glycoprotein complex playing roles in the migration and trafficking of neutrophils. Nevertheless to date nothing is known about its nuclear localization and function. In this work we investigated the interaction of β-dystroglycan with nuclear envelope protein markers and its role in the nuclear envelope organization of HL-60 differentiated to neutrophils.

Firstly, we performed immunofluorescences and immunoprecipitation assays to evaluate the colocalization and interaction between β-dystroglycan and nuclear envelope protein markers in differentiated and non-differentiated HL-60 cells. Confocal analysis revealed that β-dystroglycan is present in the cytoplasm, nucleoplasm and markedly in the nuclear envelope of HL-60 cells. Nuclear envelope protein markers such as Lamin A/C, Lamin B1, Emerin, SUN 2 and Syne 1 exhibited the characteristic distribution in the nuclear envelope. Double imunofluorescences showed a colocalization between β-dystroglycan and Lamin A/C, Emerin and Syne 1. Immunoprecipitation and western blot assays showed that Emerin, Lamin A/C, Syne 1 but not Lamin B1 and SUN 2 interacts with β-dystroglycan in nuclear extracts of HL-60 cells. Until now our results suggest that β-dystroglycan is a protein that could be involved in the organization of the nuclear envelope of the HL-60 cells.

# Mitochondrial training supercomplexes in *Ustilago maydis* cultivated with different carbon and nitrogen sources.

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The basidiomycete *Ustilago maydis* infects corn, both wild (*Zea mexicana*) and commercial (*Zea mays*) species, producing what is known as corn smut. In México, it is known as huitlacoche. This organism has been used as a model for plant- pathogen interactions and for biotechnological studies. We are interest in the composition and structure of *U maydis* respiratory chain. This organism has the four classic mitochondrial complexes (complex I, II, III, and IV) and three additional components: alternative NADH dehydrogenases, an alternative oxidase (Aox1), and glycerol-3-phosphate dehydrogenase.

In the laboratory *U. maydis* cells grows in rich medium (YPD) with a doubling time of 2.5 hours and reaches the stationary phase between 20-24 hours. However, in minimal media (MM) with glycerol or lactate as carbon sources, the cells required 150 hours to reach the stationary phase with a doubling time of 20- 23 hours; in addition, we observed morphological changes of the yeast. Previously we described the presence of individual respiratory complexes and several supercomplexes in mitochondria of *U maydis* grown in YPD. In this work we analyzed the presence of supercomplexes in mitochondria of cells grown in MM with different carbon and nitrogen sources.

Results: mitochondria isolated from cells respond correctly to the addition of ADP, cyanide and n-octylgalate. In mitochondria of cells grown in YPD or MM-Glucose and MM-Lactate the same pattern of supercomplexes were observed. In mitochondria from cells cultured in MM-Lactate one additional band of 200 kDa with activity of NADH dehydrogenase was found. Furthermore, in mitochondria from cells grown in MM-Glucose there was a band of 750 kDa associated with the activity of the complex II, suggesting the formation of a supercomplex that contains the succinate dehydrogenase.

The presence of the ATP synthase (CV), both monomeric and dimeric, was also observed. However, in samples from MM- Glycerol and MM-Lactate the activity of the dimer was very small, although the amount of dimer and monomer was nearly the same. In conclusion, mitochondria of *U. maydis* grown in different carbon sources and/or nitrogen contain the same supercomplexes but with different relative activities.

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# Electron transport chain and cellular redox state in a thermotolerant yeast

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Yeasts capable of growing and surviving at high temperatures are regarded as thermotolerant. For appropriate functioning of cellular processes and cell survival, the maintenance of an optimal redox state is critical of reducing and oxidizing species. We studied mitochondrial functions of the thermotolerant *Kluyveromyces marxianus* SLP1 and the mesophilic OFF1 yeasts, through the evaluation of its mitochondrial membrane potential ($\Delta\Psi_m$), ATPase activity, electron transport chain (ETC) activities, alternative oxidase activity, lipid peroxidation, and redox balance. Mitochondrial membrane potential and the cytoplasmic free $Ca^{2+}$ ions ($Ca^{2+}$ cyt) increased in the SLP1 yeast when exposed to high temperature, compared with the mesophilic yeast OFF1. ATPase activity in the mesophilic yeast diminished 80% when exposed to 40° while the thermotolerant SLP1 showed no change. The SLP1 thermotolerant yeast exposed to high temperature showed a diminution of 33% of the oxygen consumption in state 4. The uncoupled state 3 of oxygen consumption did not change in the mesophilic yeast when it had an increase of temperature, whereas in the thermotolerant SLP1 yeast resulted in an increase of 2.5 times when yeast were grown at $30^o$, while a decrease of 51% was observed when it was exposed to high temperature. The activities of the ETC complexes were diminished in the SLP1 when exposed to high temperature, but also it was distinguished an alternative oxidase activity. Our results suggest the significance in the control of the redox state of the mitochondria that is an important characteristic of the thermotolerance of the SLP1 yeast strain.

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# Biochemical Models for the kinesin kinetics

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Kinesin is a superfamily of motor proteins that execute a variety of intracellular microtubule-based transport functions, kinesin motor domains contain a catalytic core that display ATPase activity, microtubule binding and plus-end-directed motor activity. In particular, conventional kinesin (kinesin I) is a processive molecular motor that takes 8nm steps along microtubule for each ATP hydrolyzed. However the way this molecular motor walks over the microtubule is not clear at all. In order to study the kinesin kinetics, we present and discuss two different models. First we present the free-energy landscape useful to solve the Langevin stochastic movement equation that reproduce the typical trajectories for this proteins. Then a modified version of the Michaelis-Menten mechanism is presented to describe a 6 steps cycle considering the catalytic function of the kinesin as ATPase, in addition we consider presence of ADP in a competitive inhibition model and with the aid of chemical kinetics, we calculate the average translational velocity and also the stopping time of processes involving a collectivity of motors.

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# Biochemical characterization of an extracellular cellulase from *Sporothrix schenckii*

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*Sporothrix schenckii* is a pathogenic dimorphic fungus able to infect humans and other mammals. It has a mycelial saprophytic stage and it is widely distributed in nature. As others microorganisms, it degrades organic matter due to its secretion system. One of the main polymers of the plant cell wall is cellulose and it can be hydrolyzed by different cellulases produced by several microorganisms in order to obtain nutrients. In the present work we present the biochemical chraracterization of an extracellular cellulase secreted by *S. schenckii* in its mycelial form. The native molecular mass estimated was 197 kDa with a subunit of 96.8 kDa determined by SDS-PAGE. This enzime exhibited an optimum catalytic activity at pH 5.5/45°C and the isoelectric focusing displayed a pI value of 4.0. Its activity was inhibited by $Fe^{2+}$ and did not show effect by any other divalent ions or chelating agents. It showed activity towards cellobioside, laminarin, 4-MUG and *p*-NPG and it was slightly active towards 4-methylumbelliferyl β-D-cellobioside and *p*-nitrophenyl β-D-cellobioside but did not hydrolyze 4-methylumbelliferyl β-D-xyloside, 4-methylumbelliferyl β-D-galactopyranoside nor 4-methylumbelliferyl α-D-glucopyranoside. $K_m$ and $V_{max}$ values of the purified enzyme were 0.012 mM and 2.56 nmol/min/mg, respectively, using 4-methylumbelliferyl β-D-glucopyranoside (4-MUG) as the substrate and 44.14 mM and 22.49 nmol/min/mg when *p*-nitrophenyl β-D-glucopyranoside (*p*-NPG) was used.

This work was supported by Universidad de Guanajuato (ref. 434/2014) and by Consejo Nacional de Ciencia y Tecnología (CONACyT), México (scholarship granted to AHG).

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# Identification of the binding sites of the SBDS and EFL1 proteins to the ribosomal subunit 60S

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Ribosomes are the main component of the cell responsible of protein synthesis.1 This macromolecular complex is composed of two subunits called 40S and 60S. The first decodes the information contained in the ARNm while the latter forms the peptide bond between the aminoacids.2 The synthesis of ribosomes involves a complex process by several factors, such as enzymes, transcription regulators, cheperones, and nuclear export proteins, amongst other. Together they ensure the correct folding of the rRNA and assemble of the ribosomal proteins.3 Due to the complexity of ribosome biogenesis, alterations in this different diseases known as ribosomopathies.

One such disease, the Schwachman-Diamond syndrome is an autosomal recessive disease with an estimate incidence of one out of 50000 births. It´s characterized by exocrine pancreatic insufficiency, haematopoiesis alteration (process to build blood cells), leukemia predisposition, heart disorders, and bone marrow.4,5 The protein mutated in the Shwachman-Diamond Syndrome is named SBDS and together with the GTPase EFL1 they release the anti-association factor TIF6 from the surface of the 60S subunit. In this work we dissected the interaction between SBDS and EFL1 using fluorescence anisotropy. The SBDS protein is composed of three domains. We prepared constructs with the different domains of SBDS and combinations thereof with a C-terminal FlAsH tag (amino acid sequence Cys-Cys-Pro-Gly-Cys-Cys) by genetic engineering and were purified by conventional chromatography techniques. Protein interaction was followed by measuring the fluorescence of the LumioTMGreen dye attached to the FlAsH tag. Our results suggest that domain 1 and 2 of SBDS are involved in the interaction with EFL1, with domain 2 driving the interaction while interaction with domain 1 resulted from a positive cooperative effect.

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# Study of protein sulfhydration in the *Saccharomyces cerevisiae* proteome

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Over the last years, hydrogen sulfide ($H_2S$) has been recognized as a crucial mediator of several physiological processes in mammals. In fact, $H_2S$ due to its capacity as a signaling molecule has been proposed as a new gasotransmitter along with nitric oxide (NO) and carbon monoxide. $H_2S$ is a colorless, malodorous gas, with a high water solubility that can be cytotoxic in elevated concentrations. This gas flows freely through biologic membranes and can be produced endogenously by bacteria, archae and eukaryotes. For several years $H_2S$ has been studied for its toxic effect in several organisms, it is capable of induce reactive oxygen species production, low gluthatione levels and inhibit mitochondrial respiration. Nevertheless, $H_2S$ has been reported as an important cardiovascular regulator and neuromodulator in several *in vitro* and *in vivo* studies.

$H_2S$ mediates its effects through a protein post-translational modification: cysteine residues of target proteins can suffer a S-sulhydration in which free sulfhydryl (-SH) from a sulfhydryl donor ($H_2S$) is transferred to cysteine sulfhydryls, forming a covalent persulfide (-SSH). This protein sulfhydration affects protein function in a positive o negative way. In budding yeast, its well known that these organisms are $H_2S$ producers through sulfate assimilation pathway that includes specific transporters, permeases and sulfate reductases in order to synthetize organic sulfur metabolites. During continuous culture, $H_2S$ is capable of cell synchronization in ultradian oscillations and it is conceivable a role of $H_2S$ in glucose metabolism.

To date, there is no molecular study that addresses the biological role of endogenous production of $H_2S$ in yeast. In this work, we use the biotin switch assay in which persulfides groups (-SSH) can be detected by treatment with biotin-pyridyldithio-propionamide (biotin-HPDP), a compound that adds a biotin molecule to –SSH. Total protein extracts from yeast cultures treated with sodium hydrogen (NaHS), a $H_2S$ precursor, were obtained and incubated with methyl methanethiosulfonate (MMTS), a free thiol blocker and then exposed to biotin-HPDP for 3 hr. Immunoblots were performed using an antibody against biotin. Also, cells were grown in different culture media with or without nitrogen or carbon sources and protein extracts were obtained. In our results, we observed that a large number of proteins are basally persulfhydrated in budding yeast, after NaHS treatment the number of sulfhydrated proteins increased slightly. In order to identify protein targets of $H_2S$, streptavidin magnetic beads were used to isolated biotinylated proteins and carried out mass spectrometry. Our results showed that several enzymes of glycolytic pathway are persulfhydrated in fermentative conditions and in presence of NaHS. This post-translational modification is been evaluated in order to elucidate the role of $H_2S$ in yeast metabolism.

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# V-ATPase localization before and after acrosome reaction in mammalian sperm

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The main goal of spermatozoa is to fertilize eggs. This role involves the migration of the sperm to the ampulla of the oviduct, encounter the egg and finally fuse together. To achieve this task, the mammalian sperm must first undergo biochemical and morphological changes known as capacitation, hyperactivation and acrosome reaction. The latter occurs in the proximity of the egg and is believed to expose proteins important for the sperm to degrade and penetrate the zona pellucida (ZP), the glycoprotein extracellular matrix surrounding the plasma membrane of mammalian oocytes, and to aid in the fusion process.

Many research groups have attempted to identify the proteins that are essential in the fertilization process in sperm and their partners in the egg. The development of gene-knockout technology has allowed them to test whether the molecular entities previously proposed as being essential were in fact important or were dispensable for fertilization. By this approach, it has been discovered and confirmed the existence of some proteins whose gene disruptions prevent fertility and others that are not essential. However, the fertilization process is very complex and the complete molecular mechanism underlying this process is far from clear.

The V-ATPase is a protein complex that is implicated in many cellular processes. Its main role is the translocation of protons across membranes driven by the energy released from ATP hydrolysis. This complex is expressed in every cell type and it has been documented that, in mouse sperm, the V-ATPase is present in both membranes (internal and external) of the acrosome, an intracellular sperm granule whose external membrane is liberated during the process of acrosome reaction. After this process, the internal acrosomal membrane is exposed and interacts with the egg. However, it is unclear whether the V-ATPase remains in the internal acrosome membrane after the acrosome reaction. Considering this scenario, once in the membrane that interacts with the oocyte, the V-ATPase could acidify the extracellular media facilitating ZP degradation. The subcellular localization of the V-ATPase has not been determined in human sperm. Moreover, it is now clear that there are significant differences between mouse and human sperm.

Two different microscopy techniques (fluorescence and electron microscopy), will allow us to determine the localization of the V-ATPase in intact and acrosome reacted human sperm. Our findings will provide a starting point for proposing new hypotheses and experiments that will lead us to better understand the molecular basis of the fertilization process.

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# Role of the cytochrome P450 *TvCyt2*, in the biological associations established by *Trichoderma virens* with phytopatogenic fungi and plants.

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Naturally plants interact with beneficial and harmful organisms, in both cases activate systemic acquired resistance (RSA) and induced systemic resistance (ISR) through Microbe-Associated Molecular Patterns (MAMP's), some of which are effector-like proteins capable of altering host-cell structure and function.

The genus *Trichoderma* includes species of filamentous fungi widely known for its mycoparasitic activity. Additionally, during the interaction with plants, activate defense mechanisms and promote plant growth and development, providing plant protection against infection by pathogens and abiotic stress. There is little information regarding *Trichoderma* effector proteins and their possible role in the establishment of their biological interactions. Our working group is interested in the identification and characterization of these proteins. From a predicted secretome, we validated by qRT-PCR the differential expression of some genes, among them *TvCyt2*, which showed a significant decrease on its expression at early time of the interaction with the plant.

*TvCyt2* encodes an enzyme classified as a monooxigenase P450. In fungi this kind of enzymes are involved in metabolic pathways related to detoxification processes and secondary metabolites synthesis.

In order to determine the possible role of this product in the biological associations established by *T. virens*, we generate an overexpressing (OE-*Tvcyt2*) and a null mutant (Δ*tvcyt2*) strains of the *TvCyt2* gene. We have analyzed by GC-MS, the metabolic profile of these strains compared to the wild strain and we performed the analysis of the antagonistic capacity of these strains on different phytopathogen fungi. Additionally we have analyzed the effect of volatile and soluble compounds emitted by these strains in the activation of defense pathways in *A. thaliana* and the effect in growth, development and protection of *S. lycopersicum* plants.

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# Hypoxia induces expression of p53 in the white shrimp *Litopenaeus vannamei*

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The p53 protein is an important transcription factor for the cellular response against various types of stresses, including hypoxia. Hypoxia induces p53 in human cells. The p53 protein prevents tumor development mainly by two mechanisms, cell cycle arrest or induction of cell death by apoptosis. Little is known about the characteristics of p53 protein in invertebrates. In the white shrimp, p53 is involved in the response against oxidative stress. Recently, an induction of cellular death by apoptosis was found in shrimp hemocytes in response to hypoxia and this mechanism is independent of p53. Therefore, it is necessary to investigate whether or not p53-mediated mechanisms contribute to the survival of shrimp in hypoxic environments. The aim of this study was to quantify the expression of p53 transcripts in hepatopancreas and gills of white shrimp *Litopenaeus vannamei* under normoxic and hypoxic conditions. p53 expression was evaluated by RT-qPCR in gills and hepatopancreas of shrimp after 3, 24 and 48 hours under hypoxic conditions (1.45 ± 0.2 mg de OD $L^{-1}$). It was found that the expression of p53 transcripts in gills is 4 times higher than in hepatopancreas under normal conditions. Also, hypoxia caused a 4.5 fold increase of transcripts in gills after 3 hours and a 2.3-fold increase after 24 hours of exposure, while in hepatopancreas there is no effect on p53 transcripts. These results show that p53 is induced and activated by hypoxia in the white shrimp in a tissue specific-manner.

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# Interactions between carbon and nitrogen influence the fermentative growth of *Saccharomyces cerevisiae*

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Nutrients are responsible for growth support and cellular maintenance, nonetheless in *Saccharomyces cerevisiae* (baker's yeast) they also act as metabolic regulators. Carbon (C) is known as the main nutrient affecting alcoholic fermentation in *S. cerevisiae*, allowing fermentation as the main pathway for energy production, even in the presence of oxygen, when high levels of fermentable sources are available (Crabtree effect). In addition, nitrogen (N) starvation can lead to sluggish or stuck fermentation, and the presence of certain N sources may enhance sugars consumption, associating in this way nutritional status with alcoholic fermentation. However, little is known about nutrients interactions and their effect on fermentation. The objective of this work was to investigate the impact of different C and N sources and their concentration, on the fermentative growth of *S. cerevisiae*. The fermentative and respiratory phenotypes of *S. cerevisiae* BY4742 was studied using the doubling time ($D_t$) to discriminate among phenotypes from 81 different treatments of a $3^4$ full factorial experimental design, with five replicates. We identified two significant ($p<0.001$) triple interactions, the first one among N concentration, and the type of C and N sources, and the second one among C and N concentration and the type of N source. Results indicate the need to investigate *S. cerevisiae* nutritional status in the context of alcoholic fermentation.

Keywords: alcoholic fermentation, *Saccharomyces cerevisiae,* nutritional homeostasis, nutrient interactions.

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# Spatio–temporal characterization of $Ca^{2+}$ fluxes induced by acrosomal pH alkalinization in mouse sperm

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Fertilization is a fundamental and convoluted process, necessary to unite two haploid gametes, the male spermatozoon and the female oocyte/egg, to produce a unique individual. $Ca^{2+}$ plays a pivotal role in fertilization participating in the main functions of mammal sperm such as maturation, motility, and acrosome reaction (AR). The AR involves the exocytosis of the acrosomal vesicle in response to different physiological and non-physiological stimuli and is essential for fertilization. The acrosome vesicle contains hydrolytic enzymes that allow sperm to break down the extracellular glycoprotein matrix surrounding the egg, is an intracellular $Ca^{2+}$ store and its luminal pH ($pH_a$) is very acidic (pH 5.3). Owing to its parallels to the endolisosomal system, the acrosome has been referred to as a lysosome-related organelle. $Ca^{2+}$ efflux and osmoregulation are key elements of the AR, however, our knowledge about the role of $pH_a$ and the molecular identity and functional role of ion channels controlling $Ca^{2+}$ efflux from this important organelle is very limited. Previous evidence from our laboratory indicated that Mibefradil (10 μM) and NNC55-0396 (10 μM), both blockers of CatSper, a sperm specific $Ca^{2+}$ channel, cause an increase in intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) and elevate $pH_a$ in mouse sperm. We also observed that at ~ 10 μM both blockers induce AR. Our findings suggest that $pH_a$ alkalization regulates acrosomal $Ca^{2+}$ transporters that release $Ca^{2+}$ leading to $[Ca^{2+}]_i$ increases essential for sperm AR. Spatio-temporal and pharmacological $Ca^{2+}$ dynamics during $pH_a$ alkalization induced by Mibefradil and NNC55-0396 were evaluated by single cell fluorescence imaging. For all experiments sperm were loaded with a fluorescent $Ca^{2+}$ sensitive probe (Fluo-3, AM) and HS (in mM: 135 NaCl, 5 KCl, 1 $MgSO_4$, 20 HEPES, 5 glucose, 10 Na lactate and 1 Na pyruvate, pH 7.4 ) medium with no added $Ca^{2+}$ ($[Ca^{2+}]$= ~1 μM). We found that Mibefradil and NNC55-0396 induce an increase in $[Ca^{2+}]_i$ in a doses–dependent manner. The lysosomotropic agent GPN (Gly-Phe-β-naphthylamide), which causes osmotic permeabilization of lysosomes through its hydrolysis by the acid hydrolase cathepsin C, triggers acrosomal $Ca^{2+}$ release. We found that the increase in $[Ca^{2+}]_i$ induced by Mibefradil and NNC55-396 was affected after incubation during 5 minutes with different GPN concentrations (0, 25, 50 and 100 μM). However, we do not know if GPN lyses the acrosome. We now have to assess the effects of GPN on $pH_a$ and acrosome integrity to understand how this drug releases acrosomal $Ca^{2+}$. Additionally, we demonstrate that the Mibefradil and NNC55-396 induced release of $Ca^{2+}$ from the acrosome involves the $IP_3R$ that is present in this organelle, since Xestospongin C (1 μM), a blocker of this channel, inhibited the response.

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# Peptide profile characterization and antioxidant properties from hemp seeds (Cannabis sativa L).

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## Abstract

Hemp (Cannabis sativa L.) is an herbaceous plant in the Cannabis genus, a species of the Cannabaceae family, is one of the most versatile agricultural products on nature. Humankind has cultivated this plant along the history as a source of textile fiber, oil and food. Hemp seeds are a byproduct of industrial processing of fiber from hemp plant. Hemp seeds contained more than 30% w/w in oils and 25% w/w of high quality proteins. Nowadays there is an increasing interest on the proteins obtained from hemp seeds, due to their digestibility and satisfactory composition on essential amino acids as well as their functional properties described so far. It has been reported that hemp seeds has several properties like anti inflammatory, antiallergenic, antithrombotic, antimicrobial antineoplastic and antioxidant activity. The molecules with antioxidant activity present in

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# Ferric uptake regulator protein GDI_1248 in *Gluconacetobacterdiazotrophicus* Pal 5 strain.

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Abstract

Iron is an essential element for most organisms, including bacteria but it is biologically unavailable and plays a central role in many redox enzymes that function in electron transport chains of intermediary metabolism. Therefore, microorganisms have developed mechanisms to strictly regulate the uptake of iron. The coordinate regulation of iron acquisition systems in a lot of bacteria is mediated by a sequence specific DNA binding protein, called ferric uptake regulator (Fur). This sensing Fur protein acts mainly as a negative regulator of transcription *in vivo* by complexing with ion ferrous to repress not only the expression of iron regulated genes, but a lot of genes indirectly involved with environmental iron concentration.To date few investigations has focused to understanding the iron uptake mechanisms in *Gluconacetobacterdiazotrophicus* Pal5, nitrogen fixing endophyte isolated from sugarcane. Its genome was completely sequenced and revealed two Fur-like proteins encoded by GDI*fur*_1398 and GDI*fur*_1248. Herein, we report the functional characterize a GDI*fur*_1248 of *G. diazotrophicus,* sequence by analysis bioinformatics with other sequences, amplified the GDI*fur*_1248 coding region by PCR, cloned in TA cloning PCR2.1 Kit (Invitrogen), transformed *E. coli* TOP10 One Shot for plasmid maintenance, nucleotide sequencing (IBT-UNAM), plasmid denoted pCR*fur*_1248 , analysis by *EcoRI*restriction and PCR. After the *E. colifur* mutant H1780 (kindly donates by K. Hantke) a *fur* deficient mutant containing a fusion between the *fur* controlled promoter of the *fiu*gene and *lac*Z gene in its chromosome, were transformed pGD*fur*_1248andpCR2.1 constructions and the evaluated by*lac* phenotype and visualization of afrom white to blue on SOC media plates supplemented with 100µM FeSO4. These *in vivo* experiments suggested pGD*fur*_1248 was able to complement H1780 strain.

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# The U/DAPC is found as different assemblies as showed by BN PAGE after digitonin solubilization

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The Dystrophin / Utrophin associated protein complex (U / DAPC) is raised from membrane and cytoskeletal proteins organized structurally into three subcomplexes: dystroglycan subcomplex (Dg), sarcoglycan subcomplex (Sg) and Dystrophin (Dp) and associated cytoskeleton proteins subcomplex, which are located at the sarcolemma. The DAPC complex is expressed in striated muscle and nervous system, while UAPC is ubiquitously expressed and also may be located in smooth muscle, endothelial cells, neuromuscular junctions, etc., where Dp is replaced by utrophin (Utr). Although the U/DAPC typical function is as a molecular bridge between extracellular matrix and the cytoskeleton, also work as signaling complex which is less well known.

Here we analyze the U/DAPC by BN-PAGE in striated and smooth muscle cells after 1% digitonin treatment. We found several U/DAPC forms with heterogeneous distribution along a broad molecular weight range which could indicate different interactions or aggregation states so, the aim of this work is to determine what associations are setting the U/DAPC in those assemblies and whether these interactions affect their function (mechanical or signaling).

Materials and methods. To asses U/DAPC interactions those will be analyzed by two-dimensional electrophoresis (BN-PAGE / SDS-PAGE) and Western Blot (WB). To distinguish between complexes that have different functions a pharmacological stimulus will be applied on a smooth muscle cellular line in order to check for electrophoretic distribution profile differences on BN PAGE. U/DAPC interactions also will be verified by mass spectrometry.

We found U/DAPC co-expression in models used here. After digitonin treatment, six different forms of U / DAPC were separated, distributed as a large, medium and minimum molecular weight suggesting that these complexes have specific associations that could influence their function. 2D analysis after WB shows that these U/DAPC forms include all three subcomplexes indicating different intractions of the whole complex.

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# Sphingolipid biosynthesis and function in bacteria

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Sphingolipids exist in all eukaryotic cells, serving essential roles as structural components of cellular membranes and as signaling molecules that participate in a wide range of physiological and pathological processes. In contrast, sphingolipids occur only in few bacteria and their presence is 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 known, little knowledge exists for bacteria. An exception is the first step, catalyzed by serine palmitoyltransferase (SPT) that catalyzes a condensation between 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 to the *spt* gene, some of these operons contain a putative acyl carrier protein (*acp*) gene. This finding suggests specialized ACPs, instead of coenzyme A (CoA), are used in some cases during the initial step of sphingolipid biosynthesis in bacteria. Our bioinformatic analysis of *Caulobacter crescentus* genome shows that it contains a cluster of 8 genes, probably organized in two operons, that seem to be involved in sphingolipid biosynthesis. Specifically, in one operon, a gene encoding for a putative SPT (CC_1162) is preceeded by a gene encoding a putative ACP (CC_1163). Also, the another operon harbors a gene predicted to encode an acyl-CoA synthetase (ACS CC_1165). In our simplest model, the putative ACS CC_1165 would be in fact an acyl-ACP synthetase which converts ACP CC_1163 to its acylated form. Then, SPT CC_1162 would use L-serine and acyl-ACP CC_1163 to form 3-oxo-sphinganine, which would then be further modified to produce diverse sphingolipids. Analysis of the lipid profile of *C. crescentus* grown in minimal media at different pH (5-9), allowed the detection of candidates for sphingolipids, which could be implicated in tolerance to acid stress. Moreover, studying the biochemical function of specific genes we could show that the predicted ACP CC_1163 is an ACP, since it carries the 4'-phosphopantetheine prosthetic group. Also, the enzymatic activity of SPT from both *C. crescentus* and *Escherichia coli* BL21 (DE3) was studied, using SPT from *Sphingomonas wittichii* as positive control. After expression of *spt* candidate genes in *E. coli*, *in vitro* formation of 3-oxo-esfinganine was observed with cell-free extracts, in addition to other lipid compounds that are possibly sphingolipids. Additionally, the predicted ACS CC_1165 is able to acylate CoA. However, acylation of the special ACP CC_1163 by CC_1165 could not be demonstrated so far. Acyl-ACP synthetase (AasS) from the bioluminescent *Vibrio harveyi* strain B392 will be used to synthetize the acylated forms of special ACP CC_1163 and AcpP CC_1677 from *C. crescentus* to determine which is a better thiol substrate for the SPT CC_1162.

# FUNCTIONAL EXPRESION AND BIOCHEMICAL AND KINETIC CHARACTERIZATION OF THIOREDOXIN (Trx) AND GLUTAREDOXIN (Grx) PROTEINS FROM *Taenia solium*.

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Thioredoxin and glutharedoxin are members of the cell redox-systems involved in detoxification, enzymatic catalysis and redox signaling. Thioredoxin system includes Trx and thioredoxin reductase (TrxR) whereas Grx and glutathione reductase (GR) belong to the Grx system. Cysticerci of *Taenia solium* in the human central nervous system develop neurocisticercosis (NCC). NCC is a complex host-parasite relationship because of the presence of symptomatic and asymptomatic cases. In asymptomatic cases *T. solium* must be able to regulate high as well low levels of toxic oxidants, in the former as detoxify mechanism, in the last one as second messengers. There is not information about the presence of Trx and Grx in *T. solium* and we decided to search for them. Preliminary results indicated that we identified the Trx and Grx gens of *T. solium* (http://www.genedb.org) and then by RT-PCR using NdeI (5'- forward) and XhoI (3'- reverse) restriction sites plus total RNA both gens were expressed in pET23a. Their expressions were induced overnight with IPTG and protein purification was performed using immobilized metal-affinity chromatography (IMAC) with a Profinity Ni21- charged resin (Biorad). Grx has a 12.6 KDa corresponding with 31 pb and 105 amino acid residues. Also it was obtained from cytosolic and mitochondrial Trxs with 12.6 and 16.6 kDa respectively. All these proteins will be biochemical and kinetic characterized.

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# Purification, thermal stability analysis and crystallization trials of the human protein G0S2.

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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. To date, 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. That is why, the elucidation of the 3D structure of G0S2 would be a significant contribution to address issues specifically on the regulation of ATGL, as well as their participation in important aspects in lipolysis and other cellular processes. In order to obtain the experimental three-dimensional structure of G0S2, we expressed human G0S2 (hG0S2) as a fusion protein with SUMO protein of *S. cerevisiae* in *E. coli* with the expression vector, pETSUMO. The fusion protein SUMO-hG0S2 was found to be soluble and its purification was carried out using IMAC, ion exchange and gel filtration chromatography, the hG0S2 was released by proteolysis with PPS. We obtained protein concentrations of 10 and 5 mg/mL for the SUMO-hG0S2 and hG0S2 proteins, respectively, which allowed us to set the first crystallization trials. Finally, as part of our strategy the Thermofluor method was used to increase protein stability and its propensity to crystallize.

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# "Study of Bacillus thuringiensis Cry1Ab and Cry1Ac protoxins interaction with cadherin-like receptor from *Manduca sexta*"

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The Gram-positive bacterium Bacillus thuringiensis (Bt) produces insecticidal crystal proteins (Cry toxins) to control insect pests. Cry toxins are recognized as pore forming toxins that kill larval epithelium midgut cells by causing an osmotic shock leading to cell lysis. To induce the pore formation of Cry toxins, the parasporal crystals have to be ingested by susceptible larva, solubilized by the pH conditions of the insect gut, and activated by midgut proteases to yield the resistant core of the activated toxin. In the case of Cry toxins that are active against lepidopteran insects, it has been shown that Cry1A toxins undergo a sequential binding mechanism with glycosyl-phosphatidyl-inositol anchored proteins such as alkaline phosphatase (ALP) or aminopeptidase-N (APN) and cadherin-like protein resulting in the formation of a pre- pore oligomeric structure that is proficient in membrane insertion and pore formation. Receptor recognition by Cry toxins has been recognized as a key step of Cry toxicity that is fundamental for insect specificity. Previously we reported that Cry1Ab protoxin or activated toxin bind cadherin-like receptor with similar affinities and two different pre-pores are produced depending on which of these molecules interacts with cadherin in the presence of insect midgut proteases. Here we test the interaction with a second protoxin the Cry1Ac that share 85% of identity with Cry1Ab and both have similar toxicity against Manduca sexta larva. However, we observe that Cry1Ac protoxin has no interaction with cadherin-like receptor as Cry1Ab, our results suggest that another protein may act as receptor for Cry1Ac protoxin.

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# *Arabidopsis thaliana* mitochondrial primase helicase: biochemical characterization and protein interaction with organellar polymerases of plants

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DNA replication is carried out by common proteins present in all organisms, among these proteins are DNA polymerase, primase, helicase, and single stranded binding protein. Plant organellar (mitochondria and chloroplast) DNA replication is not a well characterized process, moreover, the proteins involved in the mechanism are still unknown. The mitochondrial primase helicase found in *A. thaliana* is believed to be the primase involved in mtDNA replication, this protein is closely related to T7 bacteriophage gene protein 4 and presents both, primase and helicase activities, also the interaction with the two organellar polymerases (POP's) present in mitochondria has not been explored. In this work, the ability of the protein to produce RNA primers on a template of ssDNA and its capacity to recognize a triple nucleotide sequence were evaluated; the interaction between AtPolIA and AtPolIB was determinated by pull-down assays, also we analyzed the capacity of the protein to shift its zinc atom to iron at his zinc finger domain and protein crystallization assays were performed in order to determinate the structure of the protein.

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# The Role of *caa*$_3$ and *aa*$_3$ Oxidases in Supercomplex Formation on the Respiratory Chain of *Bacillus subtilis*

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*Bacillus subtilis* is a Gram positive bacterium with a branched respiratory chain, in which their respiratory complexes associate in supercomplexes. Garcia et al, (2012), identified a megacomplex $b_6c$+$caa_3$+$c_{550}$ and the supercomplexes SDH+nitrate reductase (NAR), $b_6c$+$caa_3$+ATP synthase, SDH+$aa_3$ and $aa_3$+ATP synthase. Sousa et al in 2013, confirmed the SDH+NAR supercomplex and proposed a $b_6c$+$caa_3$ megacomplex that distributes electrons with high efficiency. In our works, by ion exchange chromatography, supercomplexes $b_6c$+$caa_3$ and SDH+NAR were confirmed, and evidence of the supercomplexes SDH+$aa_3$ and $b_6c$+$caa_3$+ATP synthase were obtained.

Here we report the comparison between the *B. subtilis* mutant LUW46 (*ΔqoxABCD*::Kan) that lacks the $aa_3$ and the wild type 168, analyzed by ion exchange chromatography to expose the supercomplexes $b_6c$+$caa_3$+$c_{550}$ and SDH+NAR, and to confirm the existence of the supercomplexes SDH+$aa_3$ and $b_6c$+$caa_3$+ATP sintasa.

The lack of $aa_3$ oxidase did not affect growth in 3% succinate, the total concentration of type *a* cytochromes was reduced 75% in the mutant when compared to the WT-168. Therefore we deduced that 25% of the contribution in the spectrum is from cytochrome *c* oxidase $caa_3$.

The supercomplex $b_6c$+$caa_3$+$c_{550}$+$c_{551}$ was isolated by ion exchange chromatography after solubilization with DDM in the mutant and the WT and analyzed by In-gel activity staining in 1D gels and subsequently by 2D gels. We found that it is very stable and difficult to dissociate. The supercomplex had a molecular mass between 400-1900 kDa in the WT and the mutant. The $c_{550}$ and $c_{551}$ cytochromes were strongly associated in the supercomplex so they seem to play an essential part in the supercomplex. Moreover, a fraction containing only *c*-type cytochromes was obtained in 168, while it was missing in the mutant. Lack of $aa_3$ caused that the supercomplex $b_6c$+$caa_3$+$c_{550}$+$c_{551}$ was tightly associated in LUW46 compared to the WT. We found higher molecular weight bands corresponding to the supercomplex $b_6c$+$caa_3$+$c_{550}$+$c_{551}$ in the mutant, in comparison to 168 by in-gel catalytic staining. We propose at least eight different stoichiometries for this supercomplex. In the mutant, the $caa_3$ and $b_6c$ as the supercomplex are enough to support growth, generating the proton gradient in the absence of the $aa_3$, and even quinol oxidases *bd'* type and *bb'* could take part in the electron transport chain. The SDH complex was not found associated with the quinol oxidase $aa_3$ as had been assumed but is related to the complex $b_6c$ and the, $c_{550}$ and $c_{551}$ cytochromes. The type 2 NDH does not form associations with any of the other respiratory complexes.

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# A NEW THIOREDOXIN REDUCTASE WITH ADDITIONAL GLUTATHIONE REDUCTASE ACTIVITY IN *Haemonchus contortus*

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We report herein the purification to homogeneity and the biochemical and kinetic characterization of HcTrxR3, a new isoform of thioredoxin reductase from *Haemonchus contortus*. HcTrxR3 was found to have a relative molecular weight of 134,000, while the corresponding value per subunit obtained under denaturing conditions, was of 67,000. By peptide mass spectrophotometric analysis HcTrxR3 was determined to have 99% of identical with the *H. contortus* HcTrxR1 although, and most importantly, they are different in their amino acid sequence in two amino acid positions: 48 (isoleucine instead of leucine) and 460 (leucine instead of proline). The enzyme catalyzes NADPH-dependent reduction of 5,5´-dithiobis(2-nitrobenzoic acid (DTNB) and, but also unexpectedly, the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) using NADPH as the reducing cofactor. Enzyme $K_{cat}$ values for DTNB, GSSG and NADPH were 12, 3 and 8 $s^{-1}$, respectively. HcTrxR3 developed, into specific TrxR substrates: ebselen and sodium selenite, with activity at 0.5 and 0.068 (U/mg) respectively; and 0.044 (U/mg) for S-nitrosoglutathione (GSNO) through its GR activity. The enzyme was inhibited by the gold compound auranofin (AU), a selective inhibitor of thiol-dependent flavoreductases. Although HcTrxR3 has TrxR and GR activity as thioredoxin glutathione reductase (TGR) does, it is a TrxR because it has no glutharedoxin domain and did not develop any hysteretic behavior as does TGR. The importance of this new enzyme is potential to further clarify the detoxification and haemostasis redox mechanism in *H. contortus* and also could be a protein model to recognize more differences between TrxR and GR.

The present study was funded by grant from the Universidad Nacional Autónoma de México, DGAPA (PAPIIT IN202816).

# Partial chemical characterization of zapote fruits (*Diospyros digyna* and *Diospyros rekoi*) sampled from different regions of western Mexico.

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**Objectives and methodology.** The results obtained from a partial chemical evaluation of fruits of two "zapote" species, namely *Diospyros digyna* ("zapote negro") and *Diospyros rekoi* ("zapotillo negro"), is presented. "Zapote" fruits were collected from an agro-forestry development in Taretán, Michoacán, whereas "zapotillo" fruits were obtained from the wild in Teocuitatlán de Corona, Jalisco. In both cases, fruits were collected randomly from five different trees during two successive fruiting seasons. Extracts from lyophilized fruits were analyzed to determine the levels of non-structural carbohydrates (NSCs) (i.e., glucose, fructose, sucrose and starch) through enzimatic assay and soluble protein with a Bradford comercial test. Non-targeted metabolite profiles were obtained using a DIESI-MS approach, whereas citric acid and total soluble solids (TSS) levels were determined by refractometry using fresh fruit extracts. These were also used to determine their respective pH values.
**Results and conclusions.** The NSCs assays showed no differences in sucrose and starch levels between both species. However, fructose levels were 38% higher in "zapotillo negro" fruits, while "zapote negro" fruits accumulated 31% higher glucose levels. No significant differences ($p \leq 0.5$) in NSCs levels were detected between the two sampling seasons. Protein content was 5% higher in "zapote negro" fruits. The analysis of the 100 most abundant metabolites detected by DIESI-MS indicated that the metabolic profiles in zapote fruits were defined by species and sampling season. No differences in citric acid contents between species were found. SST and pH values were within the range usually found in commercial fruits. These results suggest that fruits of both "zapote" species have the potential to be become economically important crops.

**Key words:** Non-structural carbohydrates, *Diospyros,* citric acid, metabolic profiles

# MAD2γ in the mitotic checkpoint and its association with taxol resistance in the colorectal cancer cell line HCT116

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Abnormal chromosome segregation plays a key role in cancer development. MAD2α is a component of the mitotic checkpoint (MC), a cell cycle control mechanism that ensures an accurate segregation of chromosomes during mitosis. Changes in MAD2α expression have been associated with chemo-resistance both to spindle inhibitors and to DNA damaging agents. Also, a previous study has shown that the exogenous expression of MAD2β, a splicing variant of MAD2α, was associated with resistance to Adriamycin and Vincristine in gastric cell lines. Additionally, we have previously identified a new isoform, MAD2γ, whose exogenous overexpression upon taxol-induced MC activation in the colorectal cancer cell HCT116, reduces drug-induced mitotic arrest. These findings suggested a possible structural interaction of MAD2α isoforms with MC components. To determine possible structural interactions between MAD2 isoforms and key MC components (i.e. MAD1 and CDC20), we performed an *in silico* analysis of MAD2α isoforms, Interestingly, we found that alternative splicing of MAD2α generates a premature stop codon and a frameshift in exon 4 in MAD2γ and MAD2β. This change generates a new C-terminal region in MAD2γ and MAD2β isoforms that comprise 16 amino acids, which are not present in the major isoform (MAD2α). We aligned this region with the amino acid sequence of CDC20 and MAD1 from various species and identified a MAD2-interacting motif (MIM) and a kinetochore-localization motif RLK. This finding suggests that MAD2α isoforms localize in kinetochores and may interact with the active conformation of MAD2 (C-MAD2α). We further corroborate this hypothesis by immunofluorescence. We also observed that MAD2γ overexpression in HCT116 cell line was associated with minor taxol sensitivity. Since MAD2α isoforms and CDC20 may compete for the same region in MAD2α, we propose a new model whereby MAD2α isoforms inhibits MC by interfering with C-MAD2α/CDC20 formation. This model helps to explain previous results where MAD2α isoforms overexpression seem to have an opposite role in MC signaling, and highlights the importance of studying splicing variants of MC components.

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# The conversion of a $K^+$-dependent to a $K^+$-independent pyruvate kinase and its gradual improvement in its catalytic efficiency by the contribution of three residues.

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In a phylogenetic study of the family of pyruvate kinase, it was reported that half of the sequences posses Glu117 and the other half posses Lys (according to rabbit muscle sequence (Oria-Hernández, J *et al.* (2006) *J. Biol. Chem.* 281, 30717-30724). The activities of pyruvate kinases that have Glu117 are $K^+$-dependent; those with Lys117 are $K^+$-independent. Pyruvate kinases that have Glu117 have Thr113, Lys114 and Thr120 in 97, 89 and 81% of 230 sequences, respectively. Those that have Lys117 co-evolute with Leu113, Gln114 and (Leu, Ile, Val) 120 in 97, 82 and 93% of 230 sequences, respectively. Thr113 coordinates $K^+$ in the active site, whereas residues 114 to 120 are localize in a hinge bending region that participates in the closure of the active site and the acquisition of the active catalytic conformation of the enzyme. The conservative changes between position 117 and 113, 114 and 120 suggest that these residues play a relevant role in the conversion between dependent and independent pyruvate kinases. The single mutations have been studied and reported elsewhere (Ramírez-Silva, L. *et al.* (2014) *Int. J. Mol. Sci.* 15 (12):22214-22226). In this respect, it was found that either the single T120L mutant or any mutant that contains the mutation T120L is almost or completely inactive. Therefore, in this study we explored the contribution of the addition of residue 113 or 113 and 114 to residue 117 in the expression of the $K^+$-independent activity of rabbit muscle pyruvate kinase; i.e. the mutants E117K, T113L/E117K and T113L/K114Q/E117K were studied. As previously reported, the substitution of Glu for Lys is enough to convert a $K^+$-dependent into a $K^+$-independent pyruvate kinase, although with 13% of the wild type activity. The addition of Leu113 followed by the addition of Gln114 improves its activity to about 28% and 40% of that of the wild type enzyme, respectively. Therefore, the signature L113/Q114/K117 is important to express high $K^+$-independent activities of pyruvate kinase.

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# Comparison of production and efficiency of MDMX Polyclonal Antibodies made in Rabbit and Chicken

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MDMX is a proto-oncoprotein of great biological relevance due to their ability to regulate the p53 tumour suppressor. MDMX is able to bind the p53 protein in the N-terminal region and block its transactivation activity. Its paralogous protein MDM2 is able to ubiquitinates p53 but the presence of MDMX enhance the poly-ubiquitination, then works in collaboration with MDM2 to degrade p53 by forming a heterodimer through their RING domains to down-regulated p53. After DNA damage conditions, ATM phosphorylates MDMX in serine 403; this event switch from negative to positive regulator of p53 by binding to *p53* mRNA. The commercial antibodies available are unspecific and expensive. The need for high quality antibodies from diverse sources is essential for research on this protein so important in the regulation of the main tumour suppressor protein. Here we show the overexpression and purification of specific domains of MDMX to be used to produce rabbit and chicken polyclonal antibodies. We compare their efficiency and specificity in different techniques used in our laboratory.

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# Silencing of the hypoxia inducible factor -1α decrease the white spot syndrome virus infection in shrimp muscle

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The white spot syndrome virus (WSSV) is an extremely lethal and contagious shrimp pathogen. The hypoxia inducible factor 1α (HIF-1α) is a transcription factor regulator of cell metabolism and cellular adaptation to low oxygen stress. Recently, HIF-1α has been discovered to function as a global regulator in innate immunity and infection in human. Nevertheless, the HIF-1α function during WSSV infections is not defined. Therefore, the aim of the present study was to evaluate the WSSV infection and mortality in HIF-1α knockout shrimp. The shrimp mortality were of 100% after the second day post-infection with the WSSV compared to healthy shrimp, while HIF-1α knockout shrimp survived showing good healthy. The virion copy number not were detected in muscle of HIF-1α knockout shrimp after first day post infection compare to shrimp infected, while virion copy number decreased 100- and 6- fold after second and third day post infection. Our results show that HIF-1α knockout obliterates WSSV infection suggesting that HIF-1α participate in the antiviral response of shrimp. Therefore, HIF-1α could be used as target protein against WSSV infection.

# Bionformatic analysis of genes transcription on conditions of chronological aging

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In the last years has begun the study of the molecular basis involved in the aging process, which has allowed elucidating highly conserved the ways evolved during aging process in different organisms. The processes involved are telomere shortening, loss of proteostasis, genomic instability, mitochondrial dysfunction, among others. Currently the mitochondrial dysfunction is considered as one of the main causes of aging due to the progressive accumulation of reactive oxygen and nitrogen species (ROS / RNS) which cause decreased of the functioning mitochondria. As a result the electron transport chain and oxidative phosphorylation become inefficient, decreasing production of ATP. Situation that increase susceptibility to mitochondrial ROS/RNS this lead oxidative stress, aging and finally cell death. Therefore in this work we did the bioinformatic analysis of transcripts of genes that change their expression in the aging process we were done using the PANTHER and DAVID Bioinformatics Resourses programs, with data from a expression microarray of *S. cerevisiae* RNA from cells aged *S. pombe*. Data were analyzed using the *S. cerevisiae* and *H. Sapiens* genome, finding decrease and/or increase in mRNA levels related to metabolic processes (293down/411up), development (111down/ 173up), location (129down/160up), apoptosis (47down/49up) and stimulus response (94down/132up). Inside of the process was found to increase the level of transcripts genes encoding to methionine aminopeptidase 2, caspase 3, E3 ubiquitin ligase protein XIAP, BCL2, actin, gamma tubulin, IGF2, among others. On the other hand, the decrease of mRNA levels of the genes encoding to the aminopeptidase N, lysosome associated glycoproteins, BAX, IGF2 binding protein, cytochrome C assembly protein COX 11, and apoptotic peptidase activating factor 1. As concludes that during aging deregulated are a lot of processes due to the decrease or increase of proteins necessary to maintain cellular homeostasis such as apoptosis, carbohydrates and amino acids metabolism.

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# Transcriptional modification of Fma1, Fma2 y Pca1 genes in the model of chronological aging of *Schizosaccharomyces pombe*

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Aging research has experienced an unprecedented advance over recent years, particularly with the discovery that the rate of aging is controlled, by genetic pathways and biochemical processes conserved in evolution. The study of the molecular basis of aging has allowed to elucidate some of the process that represent common denominators of aging in different organisms, such as genomic instability, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, among others. To maintain cellular homeostasis during aging is necessary that regulation processes of cell cycle and apoptosis are finely coordinated, as well as the suitable protease activity for the correct processing of nascent proteins. Has been identified that proteins as methionine aminopeptidase 1 and 2 (MAP1 and MAP2) are involved in cell cycle regulation, on the other hand, partipation of Metacaspase 1 in the apoptosis process; In this study, through PCR Real Time screening, we analized the mRNA levels of FMA1, FMA2 y PCA1 genes in the yeast *Schizosaccharomyces pombe* with chronological aging. We used yeast with 2 days (young cells) and 6 y 8 days of culture (aged cells). In the analysis of proteins involved in protein processing and regulator of cell cycle, in this work it was observed that whit 8 dyas of culture the level of FMA1 mRNA decreases while the FMA2 mRNA increases, so we can infer that the increase of FMA2 could be to compensate the dicrease of FMA1, because it has been identified that the activity of MAPs is esencial for viability and growth cell, being more serious the effect of MAP1 deletion, because when the MAP1 or MAP2 activity are inhibited this leads to apoptosis, by the cell cycle desregulation. Regarding the monitoring of apoptosis, we observed that PCA1 mRNA levels are decreased from 6 days in culture, so we can infet that the apoptosis process is dysregulated leading to a stress state in the cells population that eventualy it leads to aging.

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# Betaine Aldehyde Dehydrogenase expression during physiological cardiac hypertrophy in Sprague-Dawley rats

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Betaine Aldehyde Dehydrogenase (betaine aldehyde: NAD(P)+ oxidoreductase, E.C. 1.2.1.8; BADH) catalyze the irreversible oxidation of betaine aldehyde (BA) to glycine betaine (GB), and is essential for polyamine catabolism, γ-aminobutyric acid synthesis, and carnitine biosynthesis. GB is an important osmolyte that regulates the homocysteine levels, contributing to a vascular risk factor reduction. In this sense, distinct investigations describe the physiological roles of GB, but there is a lack of information about the GB *novo* synthesis process and regulation during hypertrophy. In this work, the BADH mRNA expression and activity were quantified in the left ventricle before, during, and after pregnancy. Both mRNA expression and protein content of BADH increased 2.54 and 2.40 fold during early and late pregnancy compared to not pregnancy, respectively, and returned to basal levels at postpartum. Besides, the GB levels increased during pregnancy and remain at postpartum. Our results demonstrate that physiological cardiac hypertrophy induced BADH mRNA expression and activity along with GB production, suggesting that BADH participates in cardiac physiological hypertrophy adaptation during pregnancy.

**Key words**: Betaine aldehyde dehydrogenase, cardiac physiological hypertrophy, glycine betaine, carnitine.

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# Trypsin activity from larval intestine of *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae)

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*Chrysomya rufifacies*, a dipteran species of forensic relevance, is associated to cadaver degradation as part of its natural diet during the larval stage; nevertheless, also presents predatory activity in an unspecific way, which makes a supposition for a broad digestive enzymes battery to deal with organic material present in cadavers. Larvae were collected during spring season, using a hog head as a bait (*Sus scrofa*). Intestines were retired from larvae of fourth instar and were submitted to protein extraction with distillated water. Protein sample was fractionated with ionic exchange chromatography using DEAE (Sigma Aldrich®) with 0.01M Tris-HCl pH 8 with discontinuous NaCl gradient of 0.05M, 0.1M, 0.3M and 0.5 M. Samples were concentrated and desalted with ultrafiltration using a membrane of molecular cutoff of 10 kDa; then, fraction with proteinase activity was subsequently precipitated using cold acetone in ratio (v/v) 1:0.25, 1:0.5, 1:1, 1:1.5, 1:2, 1:2.5 and 1:3. Enzymatic activity was detected with hydrolysis of prototype substrate N-Benzoyl-D,L-arginine p-nitroanilide (Sigma Aldrich). Proteolytic activity was detected at pH 8. Proteolytic profile with zymography in SDS-PAGE was used to visualize the purification process. Four bands of proteinase activity were observed, that migrated together during the purification process. Relative molecular weight were between 48 and 90 kDa. Detected activity was associated to serine proteinases, specifically to trypsin-like catalytic type due to pH activity, hydrolysis of prototype substrate and to susceptibility to the inhibitor PFMS. These enzymes are part of the natural battery used by the insect to feed on decaying meat; the catalytic type and the range of molecular weights coincides with reports of other dipterans.

Keywords: digestive proteinases, cadaver, dipterans.

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# Detection of the oncogenes MDMX, MDM2 and the tumor suppressor RB In Human Blood Samples

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Retinoblastoma protein (RB) tumor suppressor is implicated in many cellular processes such as cell cycle regulation, differentiation, chromatin remodeling and mitochondrial mediated-apoptosis, among others. The absence or miss function of RB is associated with several forms of human cancer. Loss of the RB function can occur through mutation in the RB gene itself, by hypermethylation in the RB promoter, by binding of viral proteins as E7 or E1A among others. In retinoblastoma RB additionally to the RB mutations, has been reported that the oncogenes MDMX and MDM2 are overexpressed, that allow a deregulation of the p53 pathway, even when p53 is wild type. In this project we want to detect and quantified the levels of the mRNA and protein of MDMX, MDM2m p53 and RB in blood samples of patients with retinoblastoma as well as in control group in order to develop a technic to detect retinoblastoma from blood samples.

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# The effect of the flavonoid nobiletin in the Akt signalling pathway and VEGF expression in the pharynx cancer *FaDu* cell line.

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Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide. Squamous cell carcinoma arises from multiple anatomic subsites in the head and neck region; risk factors include tobacco exposure, alcohol dependence and oncogenic viruses. In Mexico, squamous cell carcinomas of the upper aerodigestive tract represent 12% of the total malignant neoplasms in head and neck. Despite advances in treatment, survival rate remains static. Dietary factors play key roles in the development and prevention of various diseases, including cancer. Flavonoids are natural polyphenols present in vegetables, fruits, tea and wine produced as a result of plant secondary metabolism. Nobiletin, a polymethoxylated flavone, is abundant in the peel of citrus fruits such as oranges, lemons, and tangerines, and it exhibits anti-inflammatory, antitumor, and neuroprotective properties. The Akt/PKB signalling pathway contributes to several cellular functions including nutrient metabolism, cell growth, transcriptional regulation and cell survival. Aberrant AKT signaling leads to malignancy, either alone or in cooperation with other genetic alterations; AKT is frequently deregulated in many types of cancer, leading to an hyperactivation in human solid tumors. Vascular endothelial growth factor (VEGF) has been identified as an endothelial cell-specific mitogen that has the capacity to induce physiological and pathological angiogenesis; In the present work, we evaluated the effects of the flavonoid nobiletin over the Akt signalling pathway in the pharynx cell line *FaDu*. Using the MTT assay, we found that this flavonoid has a cytotoxic effect because it inhibits cell survival in a dose dependent manner. We also evaluated the effect of nobiletin in cell motility using the wound healing assay. Our results show that nobiletin inhibits cell migration. The expression of some proteins was evaluated using Western Blot and we found that this flavonoid may downregulate the Akt kinases, also in a dose dependent manner. We evaluated the VEGF expression by ELISA, and found a decreased expression in FaDu cells when they were exposed to nobiletin. Our results suggest that, the cytotoxic effect of nobiletin in this cells could be due the downregulation of some clue factors,and that this flavonoid could have a potential therapeutic use against pharynx cancer.

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# DROPOUT STUDENTS OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

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Dropping out of College times is related to the design of the program, inadequate selection of students, provide unsatisfactory performance previous deficiency in the academic training of students, family, economic reasons, etc. Desertion in a matter in many cases is due to poor performance and the ability to repeat it the following year, this means economic losses for the institution, affective in the family, of self-esteem for the student, who is accompanied by a feeling of frustration. The field of Biochemistry and Molecular Biology, which is taught in the first year at the Faculty of Medicine at the National Autonomous University of Mexico (UNAM) has a high rate of difficulty and the study presented here is made a recent generation of students where the total population was of 1403 students distributed in 39 groups. The matter is divided into four blocks, at the end of each one of them is a departmental test, if the average score of them is 85%, or more, the student having accredited the matter, otherwise you must submit final exam. In this study the final accreditation was 61%. The issue that we are concerned in this work is the desertion in the different evaluations. In the four departmental reviews was growing population who defected: 48 students (3.4%) in the first; 76 (5.4%) in the second; 101 (7.2%) in the third; 133 (9.5%) and in the final examination 196 students missed representing 14% of the registered population. In conclusion, the analysis is possibly representative of what happens on other occasions, subjects, careers, universities and reflection that leads is the need for high school offers vocational guidance and form properly to the student, while the universities provide transparent information, broadcasting of programs, etc. to avoid costs to the institution and the country, and what the frustration that sometimes leads to total abandonment of studies is very important.

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# Regulatory role of ADP-PFK1 and FruBPase II on the gluconeogenesis/glycolysis fluxes in *Methanosarcina acetivorans*

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In the marine archaeon *M. acetivorans* the nutritional status regulates glycogen metabolism, gluconeogenesis and glycolysis fluxes. Also, glycolysis and gluconeogenesis pathways share most of the enzymes because they catalyze reversible reactions under physiological conditions, but the phosphorylation of fructose-6P (Fru6P) to fructose-1,6-bisphosphate (Fru1,6BP) is catalyzed by an ADP-dependent Phosphofructokinase 1 and the dephosphorylation of Fru1,6BP requires a Fructose-1,6-Bisphosphatase (FruBPase) Type I/II.

In *M. acetivorans* the transcript content of the MA3344 gene encoding a FruBPase and its activity are predominantly present in cells growing under gluconeogenic condition whereas significant lower transcript content and no enzyme activity was determined in cells growing under glycolytic conditions. Instead, the transcript content of the MA3563 gene encoding an ADP-PFK and its activity was only apparent in cells growing under glycolytic conditions. This suggested that the transcript may be not translated into protein or, if translated, the enzyme was inactive (regulation by post-translational modifications) or metabolically inhibited.

Recombinant *Ma*-PFK1 activity showed dependence on ADP but not on ATP or PPi as phosphoryl donors. *Ma*-PFK1 had higher catalytic capacity in the forward reaction (*Vmax*= 7 U/mg prot., $Km_{Fru6P}$ = 0.1 mM and $Km_{ADP}$= 0.26 mM) than the reverse reaction (*Vmax*= 0.4 U/mg prot., $Km_{Fru1,6BP}$ = 0.1 mM and $Km_{AMP}$= 0.19). ATP and AMP were mixed-type inhibitors of *Ma*-PFK1 activity.

Recombinant Ma-FruBPasa II only showed activity in the forward direction (*Vmax*=2 U/mg prot. and $Km_{Fru6P}$ = 0.1 mM). AMP>ADP> ATP resulted in the negative regulation of activity.

Using *KinasePhos* software, potential sites of phosphorylation on aminoacid sequence of ADP-PFK1 and FruBPase II of *M. acetivorans* were found in Tyr, Ser and Thr residues. In cytosolic-enriched fractions, ADP-PFK1 activity was 50% lower when was incubated with an alkaline phosphatase.

In addition to gene expression, the post-translational modifications of ADP-PFK1 could provide a long- or medium-term and steady regulation of glycolytic flux. Also, a metabolic regulation of ADP-PFK1 and FruBPase could provide a fast and transitory modulation of the glycolytic and gluconeogenic fluxes. This knowledge could be used to direct future strategies of Metabolic Pathway Engineering, where the production of biomass and energy balance is a parameter that limits biotechnological processes.

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# Maintenance of intracellular hypoxia and adequate heat shock response are essential requirements for pathogenicity and virulence of *Entamoeba histolytica*

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Amoebiasis is annually responsible for million clinical cases of dysentery and thousands of deaths worldwide. It has been demonstrated that cellular adhesion, cytotoxicity and proteolysis are functions required for virulence and pathogenicity of *Entamoeba histolytica*. However, there was no correlation between these *in vitro* functions and the early elimination of non-pathogenic *E. dispar* and non-virulent *E. histolytica* (nvEh) in experimental amoebic liver abscesses developed in hamsters. Thus, additional functions may be involved in amoebic pathogenicity and virulence.In the present study, an integral experimental assessment, including innovative technologies for analyses of amoebal pathophysiology, cell biology, biochemistry and transcriptomics, was carried out to elucidate whether other cellular processes are involved in amoebal pathogenicity and virulence. Maintenance of intracellular hypoxia along with a robust heat shock protein (HSP) response in the parasite are necessary to keep the metabolic fluxes that provide both ATP and reducing equivalents to support an adequate redox potential to survive the oxidative stress. Transcriptomic analyses of amoebae under oxidative stress ($H_2O_2$ or $O_2$) revealed upregulation of HSP genes. Incomparison with virulent *E. histolytica*, the data indicated that the main reasons for the early clearance of nvEhfrom hamster liver are decreased intracellular $H_2O_2$ detoxification rate and deficient heat shock protein expression, whereas for *E. dispar*, it is a relatively lower capacity for $O_2$ reduction. Therefore, maintenance of an intracellular hypoxic environment combined with the induction of an adequate parasite HSP response to oxidative stress are essential requirements for *Entamoeba* survival in the liver, and therefore for pathogenicity.

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# Structural and functional characterization of an amaranth seed LEA Protein.

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Amaranth, a member of *Amaranthaceae* family, has been cultivated in Mesoamerica since ancient times. Its nutritional and religious relevance was decisive for the Mexica empire consolidation, hence its use in religious ceremonies and human consumption was prohibited during the colonial period. In the last few decades, the interest in amaranth consumption was resurged not only due to its high protein content and supply of essential amino acids such as lysine and methionine, but also for its starch structure and fatty acids composition[1]. Current interest in amaranth plants is also related to its extraordinary adaptability for growing in adverse climatic conditions as well its resistance to several abiotic stresses such as heat, drought and salinity. Recently, the amaranth seed proteome of *A. cruentus* was characterized and reported by our research group[2], wherein about more than 400 proteins spots were resolved on two-dimensional gel electrophoresis (2-DE) and protein spots were identified by liquid chromatography tandem mass spectrometry. A quite distinctive protein took our attention due to its spot intensity in the 2-DE gel, this protein was identified as a Late Embryogenesis Abundant (LEA) and as far we know, is the first report of this kind of molecules in amaranth seed. LEA proteins, as the name suggest, are mainly storage in the seed during late stage of embryogenesis although are not exclusive of plantae kingdom. LEA proteins are diverse and wide distributed and its function has been related with protection mechanisms against desiccation, freeze, heat tolerance, as well high salinity but in many cases their individual physiological activity remains unknown. LEA proteins share common physiochemical and structural features, most of them are highly hydrophilic and predicted as intrinsically disordered proteins even though amino acid composition has propensity to form helical structure[3].

In order to gain knowledge into functionality and structural properties of this novel LEA, the gene was cloned (*AcLEA*) into pET expression vector and recombinant protein was purified and characterized. AcLEA function was evaluated using *E. coli* as *in vivo* model and structural characteristics were revealed by spectroscopic studies based on circular dichroism methods. Our data demonstrate that recombinant AcLEA plays and important role in the abiotic stress response in *E. coli*, conferring tolerance to desiccation and protection against oxidant conditions in prokariota cells. Additionally, in this work we show that AcLEA is intrinsically disordered in solution even at high salinity and osmotic pressures but has a strong tendency to form secondary structure, mainly folded as alpha helix, when an inductive additive is present.

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# SMALL-ANGLE-X RAY-SCATTERING (SAXS) STUDIES OF THE LOW-RESOLUTION STRUCTURE OF THE RIBOSOMAL GTPASE EFL1, THE SBDS PROTEIN AND THEIR COMPLEX

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Ribosome biogenesis is closely linked to the cell growth and proliferation. Dysregulation of this process causes several diseases collectively known as ribosomopathies. One of them is the Shwachman-Diamond Syndrome, and the SBDS protein mutated in this disease participates with EFL1 in the cytoplasmic maturation of the 60S subunit. Recently, we have shown that the interaction of EFL1 with SBDS resulted in a decrease of the Michaelis-Menten constant ($K_M$) for GTP and thus SBDS acts as a GEF for EFL1 (1). Subsequent studies demonstrated that SBDS debilitates the interaction of EFL1 with GDP without altering that for GTP (2). The interaction of EFL1 alone or in complex with SBDS to guanine nucleotides is followed by a conformational rearrangement. Understanding the molecular strategy used by SBDS to disrupt the binding of EFL1 for GDP and the associated conformational changes will be key to understand their mode of action and alterations occurring in the disease. In this study, we aim to show the conformational changes resulting from the interactions between EFL1 and its binding partners, the SBDS protein and the guanine nucleotides using SAXS technique. SAXS provided structural information of the proteins and their conformational changes.

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# The size of gold nanoparticles affects the antimicrobial activity

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*Streptococcus pneumoniae* is a Gram positive bacterium which causes otitis, sinusitis or infections more severe like pneumonia, meningitis or even worse septicaemia, resulting lethal in several occasions for children and old people. The principal treatment is based on antibiotics as beta-lactams, trimethoprim-sulfamethoxazole, or inclusive in some cases it is necessary to give antibiotics of third generation such as vancomycin. Since the excessive use of antibiotics produces resistance or multi-resistance, it is therefore imperative to develop new alternatives of treatment, one of them could be gold nanoparticles (AuNP´s). The AuNP´s of 20nm tested in a non-pathogen laboratory strain, have shown to be a good alternative if they are utilized as antibacterial. Unfortunatelly, other sizes have not been tested yet, for this reason we decided to analyse the antibacterial effect of AuNP´s whose sizes were 11, 16, 28 and 38 nm. These NP´s were re-coated with polyethylene glycol. The antimicrobial activity was tested on two strains: ATCC 49619 (capsulated) and R6 (non-capsulated). We observe an increase of antibacterial activity when the small size was tested (11 nm) in comparison with 16 nm.The minor efect was observed with the AuNP´s of 38 nm. In other words, the antibacterial efect is inversely proportional to size of AuNP´s. We think that smallest size crosses easy the wall cellular bacterial.

# Curcumin inhibits the thioredoxin-glutathione reductase (TGR) from larva *Taenia crassiceps* (cysticerci)?

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Curcumin is a polyphenol, extracted from the rhizome of the plant *Curcuma longa*, used in Asia and Europe in traditional medicine and food. Several therapeutic properties have been attributed to the use of curcumin as anti-parasitic and as an anti-cancer compound. Some of these properties are related with the inhibition of thioredoxin reductase (TrxR), an antioxidant enzyme that in its C-terminal redox center a residue of selenocysteine (SeCys) is present. In parasites, tapeworms as *Taenia solium* and *Taenia crassiceps* TrxR and GR are absent and supplied by a thioredoxin-glutathione reductase (TGR), an enzyme that could reduce glutathione and thioredoxin. TGR is the only enzyme responsible of redox homeostasis in Platyhelminths parasites and reported as a drug target against these parasites. It has been reported that curcumin inhibits proliferation of cancer cell lines as, HL-60 (leukemia) and AK-5 (colon and mamma); in these cells, the thioredoxin system (TrxR, Trx and Tpx) is over-expressed. Additionally studies, reported that curcumin binds to SecCys residue of TrxR in HeLa cells, but this inhibition is very slow (minutes).

In this work, we study the inhibition by curcumin (sigma) of purified TGR obtained from *Taenia crassiceps* cysticerci. As reported in the literature, we also found that the inhibition takes long time (2 hours). It is known that curcumine is very unstable once in solution and is degraded to different components as: vanillin, ferulic acid, feruloilmethane and ferulyc aldehyde and other small compounds. A possible explanation for this slow inhibition could be related with the presence of one (or More) of these degradation products and that inhibition occur when it appears in the sample. The inhibitory effect of commercial products obtained from curcumine degradation are presented.

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# Peroxisome/mitochondrial dynamics and development of *Podospora anserina* in a *fis1* deletion strain

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Peroxisomes are highly versatile and dynamic organelles, required for the development of several eukaryotic organisms. We have shown that peroxisomes are needed for proper development of several stages of sexual differentiation in *Podospora anserina*. In addition, we demonstrated that peroxisome biogenesis and dynamics are highly regulated during both vegetative and sexual development of this fungus. We showed that during vegetative growth most peroxisomes are propelled towards the extending hyphal tip by the cytoplasmic bulk flow, however, a small population of peroxisomes shows bidirectional long-distance movements, which allows them to reposition in the cell. Also, we demonstrated that peroxisomes change their morphology and number in response to the metabolic demands of the cell or to different environmental cues. Heat stress and growth on oleic acid as sole carbon source induced peroxisome proliferation, whereas oxidative stress or low temperature treatment promoted peroxisome elongation. Further, we observed that sexual development is also accompanied by changes in peroxisome number, distribution and morphology, which take place at specific stages. Taken together, our findings suggest a precise regulation of peroxisome dynamics is involved in modulating the development of this fungus. To test this hypothesis, we obtained mutant strains lacking the peroxisome/mitochondrion fission factor FIS1, which in yeasts provides the membrane anchor for the dynamin related protein (DNM1) that cleaves the membrane of these two organelles. Our experiments reveal that lack of FIS1 in *P. anserina* produces a dramatic increase in peroxisome and mitochondrion elongation in vegetative cells, consistent with a role for this protein in the division of both organelles. Moreover, we observed that the formation of spores during sexual development is also compromised in absence of this protein. Our data provides evidence that regulation of peroxisome and mitochondrion dynamics are important for proper development in *Podospora anserina*.

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# Purification of a type lectin protein from pericarp *Opuntia ficus-indica* that shown agglutination and cytotoxic effect

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Knowledge area: Estructura, Diseño y Función de Proteínas

Lectins are proteins or glycoproteins which bind specifically to mono- or oligosaccharides and glycoconjugates and these proteins have been purified from various plant and other organisms tissues. However, the majority of lectins have been isolated from plants. The plant lectins are involved in biological defense against pathogens, symbiotic adhesion with nitrogen fixation bacteria, fuel proteins for seed germination, etc. In addition, several applications have been described for lectins, highlighting his application to specifically recognize oligosaccharides moieties in cancer, because in these disease the cell malignant have structural alteration of carbohydrate chains and they may be involved in cancer progression and malignancy.

The Cactaceae family comprises more than 1500 species, however only the agglutinin *Macarocereus eruca* (MeA) has been isolated from cactaceae. This lectin has been shown galactose specificity and differential recognition to breast and cervical cancer cell lines. Therefore, identification and characterization for new cactaceae lectins could be tools for diagnostic and treatment of cancer. The aim of this study was to identify and purify a protein like lectin of pericarp from *Opuntia ficus-indica* that shown agglutination, sialic acid agglutination inhibition and cytotoxic effect to cervical cancer cell lines.

The preliminary results shown that protein fraction have differential agglutination activity among tumorigenic cell lines evaluated but no for the non-tumorigenic from cell line control. Moreover, this agglutination activity was inhibited in presence of sialic acid. The incubation of this protein fraction, shown a negative effect in cellular viability for all cell lines tested, including the non-tumorigenic control cell line this inhibition to related with the cell line which to prove. However, the inhibitory effect in cell viability was compared to WGA lectin used for control these effect was similar. Finally, the protein fraction which shows activity was separated through HPLC-molecular exclusion chromatography to obtain three proteins with approximate weights of 66.45, 25.5 and 18 kDa.

Key words: Purification, lectin, agglutination, cytotoxic effect, *Opuntia ficus-indica*

# Probing the phosphoryl-group transfer routes in the ArcB dimer.

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Two-component signal transduction pathways (TCS) are widespread in prokaryotes and play extensive roles in adaptation to environmental changes. The Arc (anoxic redox control) TCS is an important element in the complex transcriptional network that allows facultative anaerobic bacteria, such as *Escherichia coli,* to sense various respiratory growth conditions and adapt their gene expression accordingly. This system comprises the cytoplasmatic response regulator ArcA and the transmembrane sensor kinase ArcB. Unlike the vast majority of sensor kinases in *E. coli*, which possess just one catalytic domain, ArcB is a tripartite kinase with three phosphorylation domains.

Under anoxic growth conditions, ArcB autophosphorylates and transphosphorylates ArcA through a $His^{292} \rightarrow Asp^{576} \rightarrow His^{717} \rightarrow Asp^{54}$ 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 $Asp^{54} \rightarrow His^{717} \rightarrow Asp^{576} \rightarrow$ 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 and inhibition of Polyphenol oxidase from Hass avocado (*Persea americana*).

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The Hass avocado (*Persea americana*) belongs to the Lauraceae family, it is native to the highlands of Mexico and Central America; It is mainly used as a complement in foods, besides form its rich fat can be extracted an oil used in the cosmetic and pharmaceutical industry.

Polyphenol oxidase (PPO) is the mainly responsible of enzymatic browning in many fruits during ripening process, handling, storage and processing thereof; for this reason it is considered a critical enzyme. Reactions that catalyzes the enzyme are: hydroxylation of monophenols to *o*-diphenols (cresolase activity) and oxidation of *o*-diphenols to *o*-quinones (polyphenol oxidase activity). One of the major problems in the handling of avocados and other fruits is the short time shelf life and its strategic complications for commercialization, due to the rate of enzymatic browning, PPO provokes the loss of sensory properties.

In this study, PPO activity in crude extracts was inhibited using salicylic acid, EDTA, citric acid at different concentrations (0.1, 1 and 10 mM) besides microwave was used. Subsequently purified Hass Avocado PPO was partly characterized i.e. molecular weight, temperature and pH optima, were determined. Also affinities, were measured to different substrates using 4- methyl catechol, tyrosine, caffeic acid, L- Dopa, pyrogallol, catechol and ferulic acid; on the other hand some specific inhibitors were used: tropolone and kojic acid at different concentrations, and the same used in the inactivation crude extracts were performed.

# Claudin-6, -7 and -9 transfected AGS cells induce Hsp-27, -40, -70 and -90 protein expression.

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Gastric adenocarcinoma causes about one million deaths worldwide annually. In metastatic forms, tumor cells can infiltrate tissue lymph vessels and spread to lymph nodes and into the bloodstream, after which the road is open virtually any organ in the body.

During the development of cancer, multiple cell changes alters the expression of certain proteins, enzymes and essential cell pathways from which the tumor phenotype results. This changes enable the cells to acquire advantages that favors the development of the tumor, such as the ability to invade and metastasize; the latter require the degradation of extracellular matrix (ECM), a process that is carried out by enzymes called metalloproteases (MMPs), which have the ability to alter cell-cell or cell-ECM contacts whit the help of proteins that assist in his activation.

There is a close relationship between metalloproteinase and heat shock proteins (Hsp´s). The latter, play an important role in the migration of cancer cells and are involved in the loss of cell-cell adhesion, activation of MMPs and migration and invasiveness of malignant cells.

It has been reported that gastric adenocarcinoma cells that overexpress tight junction proteins claudins-6, -7 and -9, show an increase in proliferation, migration, cell invasiveness and expression of MMP-2 and MMP-9. Because the Hsp´s are involved in the activation of MMP-2 and -9, in this project was assessed, in a cell line of gastric human cancer such as AGS cells, if overexpression of claudins -6, -7 and -9 correlated with changes in expression and secretion of heat shock proteins (mainly Hsp27, -40, -70 and -90) and if this claudins co-localize with the Hsp´s.

The results showed an increase in expression of Hsp40 and Hsp90 in AGS cells transfected whit claudin-9. A weak co-localization of Hsp90 whit claudin-6 and -9 was also observed. The results collectively suggest that overexpression of claudin-9 may be promoting the activation of MMP-2 and increasing the expression of Hsp90. This results together with the increased cell permeability, promote the secretion of MMPs and Hsp´s through damaged paracellular pathways. Hsp´s probably participate by maintaining a proinflammatory environment (with induction and secretion of cytokines and expression of surface markers) favorable for the proliferation and invasion of cancer cells and the degradation of extracellular matrix by MMPs.

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# Evaluation of cytotoxic, antioxidant and anti-inflammatory activity of essential oil and terpenes of *Satureja macrostema*

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*Satureja macrostema* is a Mexican medicinal plant known as nurhitini té or nurite, whose antioxidant properties are mainly attributed to the volatile compounds from aerial part. Therefore, the goal of the present study was to investigate the *in vitro* cytotoxic, antioxidant and anti-inflammatory potential of essential oil and some related terpenes of *S. macrostema*. Essential oil of fresh aerial parts of *S. macrostema* plants were prepared by hydrodistillation and characterized by GC-MS, finding that the major terpenes were caryophyllene, limonene, linalool, menthone, pulegone and thymol. Antioxidant effectiveness of essential oil and terpenes of *S. macrostema* was examined by two different radical scavenging methods, DPPH (2, 2-Diphenyl-1-picrylhydrazyl) and ABTS (2, 2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid), testing 1, 10, 100 and 1000 µg/mL. The essential oil showed the highest antioxidant capacity, reaching 53.10% capture of radicals in the DPPH and 92.13% in the ABTS method. Thymol was the terpene with higher antioxidant activity (94%) at 1 µg/mL using both methods. In addition, the cytotoxic effects of essential oil and terpenes of *S. macrostema* on bovine umbilical vein endothelial cells (BUVEC) were determined. Different concentrations of essential oil and terpenes (1, 10, 100, 1000 µg/mL) were added to cultured cells and incubated for 24h. Cell survival was evaluated using the MTT-based cytotoxicity assay and trypan blue exclusion. The essential oil and linalool, menthone and pulegone did not affect the BUVEC viability (> 86%), while limonene, caryophyllene and thymol showed toxicity to 1000 µg/mL, reducing cell viability (~40% of reduction). The results obtained show that essential oil and the major terpenes of *S. macrostema* have antioxidant activity at concentrations that do not compromise BUVEC viability. From these data, we tested essential oil and terpene compounds at concentrations of 1 and 100 µg/mL to analyze the gene expression of cytokines related to the inflammatory response using real-time PCR. The gene expression of the pro-inflammatory cytokine Tumor Necrosis Factor-α (TNF-α) and the anti-inflammatory cytokine Interleukin 10 (IL-10) were analyzed in BUVEC. The essential oil at 100 µg/mL induces two times the expression of IL-10. Instead, limonene, linalool, menthone, pulegone and thymol do not exert activity on the expression of both genes (at two concentrations tested). Only caryophyllene terpene (100 µg/mL) inhibits TNF-α up to five times. These results indicate that the essential oil and terpenes of nurite exert antioxidant and anti-inflammatory effects on BUVEC under basal conditions.

Key words: Anti-inflammatory, Bovine endothelial cells, *Satureja macrostema*, Radical scavenging activity, Terpene.

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# Construction of variants of Cyt1A toxin from the *Bacillus thuringiensis* to migrate their toxicity to lepidopteran insects.

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Entomopathogenic bacterium *Bacillus thuringiensis* is widely used as a biological alternative to chemical pesticides in the control of insect populations which are either agricultural or forestry pests or to reduce populations of insects that are vectors of severe human diseases. The toxicity of *B. thuringiensis* to insects is due to the δ-endotoxins found in the parasporal crystals. These are proteins that kill the larvae since they knock down midgut cells of susceptible insects. Insecticidal δ-endotoxins are produced during the sporulation phase of growth and are classified into two families of membrane-perforating toxins, the crystal (Cry) specific to Lepidoptera and the cytolytic toxins (Cyt) specific to Diptera.

The mechanism of action of Cry toxins is a complex process which involves the binding to specific protein receptors located on the midgut cells surface, such as cadherin-like proteins (CAD), aminopeptidase N (APN) and alkaline phosphatase (ALP). The interaction with CAD is a complex interaction that involves three epitopes in the CAD corresponding to extracellular regions named CR7, CR11 and CR12, these epitopes of CAD protein interact with exposed loops 2, 3 and α-8 from domain II of the Cry1Ab toxin. The interaction with APN occurs through exposed loop 3 of domain II and the interaction with ALP through strand β-16 of domain III of the toxin. In contrast, the mechanism by which Cyt damages cell membrane is still a subject of debate. The accepted model for Cyt action suggests that the monomer undergoes conformational changes upon membrane contact, forming pores that induce colloid-osmotic lysis of the cells. An alternative mode of action, known as the detergent-like mechanism, presumes that the Cyt proteins do not perforate the membrane but rather adsorb as aggregates onto the surface, thereby causing defects in membrane packing that enable leakage of intracellular molecules. In addition, Cyt toxins enhances the activity of Cry4 and Cry11 in mosquitoes, producing a synergistic effect.

In this work, we carried on the insertion of the loop 3 of Cry1Ab in different sites of the Cyt1A toxin [Cyt1A-Loop3-addition-Loopn (CL3ALn)], to induce binding of Cyt1A toxin to the receptors APN, ALP and CAD which are present in *M. sexta* midgut cells. We constructed ten different Cyt chimeric toxins, but only seven were stable (CL3AL4, CL3AL5, CL3AL6, CL3AL7, CL3AL8, CL3AL9 and CL3AL10). ELISA assays were used to demonstrate increased binding of seven CL3ALn to receptors of *M. sexta* compared with Cyt1A wildtype. Chimeric toxin with loop 6, 7 and 9 (CL3AL6, CL3AL7 and CL3AL9) showed the highest levels of binding in ELISA assays. Our data suggest that loops 6, 7 and 9 provides the optimal sites for insertion of the loop3 of Cry1Ab into Cyt1Aa. The toxicity of these constructions will be tested against different insects pests.

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# IL-8 secreted by AGS cells modify transepithelial electrical resistance and leukocyte adhesion on HUVECs

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Tight junctions (TJs) are the most apical intercellular junctions that have an important role in physiological functions, such as ion selectivity, cell barrier against pathogens and homeostatic regulation. The transmembrane proteins called claudins have an important role in TJs. Previous reports have shown altered expression of claudins in different types of cancer. Additionally, AGS cells overexpressing claudin 6 and 9 increased invasiveness, migration and proliferation rate. The mechanisms of intra and extravasation are regulated by some proinflammatory cytokines (IL-8) decreasing transephitelial electrical resistance (TER) and the expression of TJ proteins.

The aim of this study was evaluate if cytokines in conditioned medium of claudin 6 and 9 transfected AGS cells modify leukocyte adhesion and TER of Human Umbilical Vein Endothelial Cells (HUVEC). To evaluate these, proinflammatory cytokines concentrations were determined by flow cytometry with a commercial kit using conditioned medium of control AGS and claudin 6 and 9 overexpressing AGS cells cultured at different time periods. Culture supernatants of transfected cells were used to evaluate U937 cell adhesion on HUVECs and barrier integrity using a TER automatized reader.

The results show that IL-6 and IL-10 were in the 1-4pg/ml concentrations and IL-8 was in the 100-400 pg/ml range; the differences responded to culture time. The supernatants of transfected AGS cells diminished the HUVECs TER and leukocyte adhesion compared with control AGS cells. Future experiments will determine if these cytokines modify claudins expression on HUVECs to promote the intra and extravasation process.

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# Dispensability of the [4Fe-4S] cluster in novel homologues of adenine glycosylase MutY

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## Abstract

7,8-Dihydro-8-deoxyguanine (8oG) is one of the most common oxidative lesions in DNA. DNA polymerases misincorporate an adenine across from this lesion. Thus, 8oG is a highly mutagenic lesion responsible for G:C→T:A transversions. MutY is an adenine glycosylase, part of the base excision repair pathway that removes adenines, when mispaired with 8oG or guanine. Its catalytic domain includes a [4Fe-4S] cluster motif coordinated by four cysteinyl ligands. When this cluster is absent, MutY activity is depleted and several studies concluded that the [4Fe-4S] cluster motif is an indispensable component for DNA binding, substrate recognition and enzymatic activity. In the present study, we identified 46 MutY homologues that lack the canonical cysteinyl ligands, suggesting an absence of the [4Fe-4S] cluster. A phylogenetic analysis groups these novel MutYs into two different clades. One clade is exclusive of the order Lactobacillales and another clade has a mixed composition of anaerobic and microaerophilic bacteria and species from the protozoan genus Entamoeba. Structural modeling and sequence analysis suggests that the loss of the [4Fe-4S] cluster is compensated by a convergent solution in which bulky amino acids substitute the [4Fe-4S] cluster. We functionally characterized MutYs from Lactobacillus brevis (LbY) and Entamoeba histolytica (EhY) as representative members from each clade and found that both enzymes are active adenine glycosylases. Furthermore, chimeric glycosylases, in which the [4Fe-4S] cluster of Escherichia coli MutY is replaced by the corresponding amino acids of LbY and EhY, are also active. Our data indicates that the [4Fe-4S] cluster plays a structural role in MutYs and evidences the existence of alternative functional solutions in nature.

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# Revealing the structural and energetics basis of ABL tyrosine kinase inhibition by target-directed drugs

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Under normal conditions, the catalytic activity of the tyrosine kinase Abl is tightly regulated by various auto-inhibitory mechanisms. In chronic myeloid leukaemia (CML), Abl is constitutively active through genetic fusion with Bcr. This malignant hematopoietic disease affects around 1.5 millions of people patients worldwide. Expression of Bcr-Abl protein leads to activity deregulation of the tyrosine kinase due to the loss of an N-terminal inhibitory element present in normal Abl. Imatinib is a phenylaminopyrimidine derivative that has high affinity and specificity for the catalytic site of Abl, which has proved clinically successful in the treatment of CML. However, due to imatinib resistance by point mutations in the Abl kinase domain, more than 100 new several inhibitors have been further developed, such as dasatinib, although none of them shows efficiency in all treated patients. In spite of extensive structural information, the binding energetics of Abl to drug-like inhibitors is largely unknown. Unveiling the relationship between structural information and energetic properties involved in molecular recognition processes increasingly has allowed the generation of predictive models useful in rational drug design. In this work, isothermal titration calorimetry was used to characterize, as a function of temperature, the association process of Abl to imatinib (which preferentially binds to the inactive, or DFG-out, conformation of Abl) and dasatinib (which preferentially binds to the active, or DFG-in, conformation of Abl). Our results show that the binding of imatinib to Abl is a process enthalpically driven and entropically disfavored, with a dissociation constant in the sub-micromolar range. The binding heat capacity ($\Delta Cp$) showed a strong thermal dependence, suggesting the occurrence of a conformational selection binding mechanism. In contrast, although the binding constant between dasatinib and Abl was also around in the sub-micromolar range, the process was both enthalpically and entropically driven. In this case, $\Delta Cp$ showed no dependence on temperature, and was more negative than that expected for a rigid-like association. Therefore, dasatinib induces a conformational change upon binding to Abl.

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# Metabolomic study of *Lasiodiplodia theobromae*

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Plant pathogenic fungi such as those from the Botryosphaeriaceae affect a wide variety of economically important plants, such as *Vitis vinifera* (grapevine) and *Olea europea* (olive). A particularly virulent member of this family of fungi, *Lasiodiplodia theobromae*, causes disease known as “dieback” or “black dead arm”. It has been found to infect over 500 species of plants worldwide. From a metabolomics perspective, this work identified fatty acid esters as metabolites produced by the fungus *L. theobromae* in natural substrates. This was done via the purification and chemical characterization of ethyl linoleate (LAEE), using thin layer chromatography (TLC), HPLC, mass spectrometry and nuclear magnetic resonance (NMR) and gas chromatography-mass spectrometry (GC-MS). GC-MS identified a variety of fatty acid esters produced by *L. theobromae*.. A few of these compounds showed physiological activity in the plant model *Nicotiana tabacum* during germination and early growth. LAEE, ethyl stearate (SAEE) and ethyl palmitate (PAEE) inhibited tobacco growth at high concentrations (50-200 µg/mL) and induced germination and growth at low concentrations (25 µg/ml-98 ng/mL). Experiments on the long term effects of these compounds were done on *N. tabacum* and other plants via hydroponics, whose effects on growth depended on the plant species. In *N. tabacum*, LAEE y SAEE inhibited growth and caused chlorosis after germination and one month of growth. However, PAEE induced growth, with an effect on true leaves, which grew significantly larger with 1 µg/mL PAEE. Proteomic characterization during the production of fatty acid esters (FAE) was done using *de novo* sequencing. Many novel proteins were detected in *L. theobromae*, including pathogenicity-related proteins such as Cap 20, and cyanovirin-N, a lectin with great anti-viral activity, among many other proteins of interest.

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# Study of the NAC-mediated interactions between cytosolic ribosomes and Sam37 during mitochondrial protein import

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The process of cotranslational mitochondrial import couples the translation of a polypeptide with its insertion into the organelle. Although the vast majority of the mitochondrial proteins is encoded in the nucleus and synthesized by cytosolic ribosomes, little is known about the early steps of recognition and targeting to the mitochondrial surface. Our research focuses on the Nascent-polypeptide Associated Complex (NAC), a heterodimeric cytosolic chaperone involved in recognition of nascent chains at the ribosomal exit tunnel. NAC has been involved in mitochondrial protein localization and its absence produces a misstargeting of mitochondrial proteins to the endoplasmic reticulum. At the mitochondrial surface we are studying the membrane-associated protein Sam37. Previous results from our laboratory have shown that the simultaneous deletion of *SAM37* and subunits of NAC present an aggravating genetic interaction, suggesting that NAC and Sam37 cooperate during mitochondrial protein import. In this work we evaluate the physical interactions between cytosolic ribosomes and the mitochondrial surface, in particular we focus on the interactions mediated by the NAC complex and the role of Sam37 as docking receptor at the outer membrane.

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# Evaluation of the Antimicrobial Activity of Components of the Venom of the Snake *Bothrops ammodytoides*.

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The venom of snakes is integrated by several components, including some with antimicrobial activities, *Botrhrops ammodytoides* is a snake from Argentina, in their venom some enzymatic activities as Phospholipase and Serine Protease have been reported, however the antimicrobial potential of their venom still unexplored in detail. In this work the venom of *B. ammodytoides* was fractionated by RP-HPLC, 19 different components were separated and vacuum dried, the antimicrobial activity of the all fractions was evaluated towards the bacterial strains, *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 in agar plates. None of the fractions showed antimicrobial effects on the Gram positive bacterial strain *S. aureus* ATCC 25923, however all components isolated from the venom displayed a bactericidal effect over the Gram negative bacterial strain *E. coli* ATCC 25922. With the aim of identify if the antimicrobial components were enzymatic or peptidic, a SDS-PAGE acrylamide gel was prepared, none component with an apparent mass weight under 10 KDa was observed, so that the antimicrobial activity was not related to a peptide in the venom, were observed bands around 15-20, 35 and 80 KDa, suggesting that the antimicrobial effect was related to an enzymatic activity, six fractions with bands in the 15-20 KDa range were tested to evaluate Phospholipase A2 activity, of the six fractions evaluated five showed a Phospholipase A2 activity, the antimicrobial activity of the *B. ammodytoides* venom could be related to this activity, in several reports Phospholipase A2 type enzymes from venoms of snakes of the *Bothrops* family, display antimicrobial activities toward different bacteria.

**Mitochondrial training supercomplexes in *Ustilago maydis* cultivated with different carbon and nitrogen sources.** Deyamira Matuz Mares, Héctor Vázquez Meza, Juan Pablo Pardo Vázquez. C. P. 04510, Tel: 56232168, Fax: 56162419; pardov@bq.unam.mx. Department of Biochemistry, Faculty of Medicine, UNAM, Av. Universidad 3000.

The basidiomycete *Ustilago maydis* infects corn, both wild (*Zea mexicana*) and commercial (*Zea mays*) species, producing what is known as corn smut. In México, it is known as huitlacoche. This organism has been used as a model for plant- pathogen interactions and for biotechnological studies. We are interest in the composition and structure of *U maydis* respiratory chain. This organism has the four classic mitochondrial complexes (complex I, II, III, and IV) and three additional components: alternative NADH dehydrogenases, an alternative oxidase (Aox1), and glycerol-3-phosphate dehydrogenase.

In the laboratory *U. maydis* cells grows in rich medium (YPD) with a doubling time of 2.5 hours and reaches the stationary phase between 20-24 hours. However, in minimal media (MM) with glycerol or lactate as carbon sources, the cells required 150 hours to reach the stationary phase with a doubling time of 20- 23 hours; in addition, we observed morphological changes of the yeast. Previously we described the presence of individual respiratory complexes and several supercomplexes in mitochondria of *U maydis* grown in YPD. In this work we analyzed the presence of supercomplexes in mitochondria of cells grown in MM with different carbon and nitrogen sources.

Results: mitochondria isolated from cells respond correctly to the addition of ADP, cyanide and n-octylgalate. In mitochondria of cells grown in YPD or MM-Glucose and MM-Lactate the same pattern of supercomplexes were observed. In mitochondria from cells cultured in MM-Lactate one additional band of 200 kDa with activity of NADH dehydrogenase was found. Furthermore, in mitochondria from cells grown in MM-Glucose there was a band of 750 kDa associated with the activity of the complex II, suggesting the formation of a supercomplex that contains the succinate dehydrogenase.

The presence of the ATP synthase (CV), both monomeric and dimeric, was also observed. However, in samples from MM- Glycerol and MM-Lactate the activity of the dimer was very small, although the amount of dimer and monomer was nearly the same. In conclusion, mitochondria of *U. maydis* grown in different carbon sources and/or nitrogen contain the same supercomplexes but with different relative activities.

This work was partially supported by PAPIIT IN209614

# Mitochondrial training supercomplexes in *Ustilago maydis* cultivated with different carbon and nitrogen sources.

Deyamira Matuz Mares, Héctor Vázquez Meza, Juan Pablo Pardo Vázquez. C. P. 04510, Tel: 56232168, Fax: 56162419; pardov@bq.unam.mx. Department of Biochemistry, Faculty of Medicine, UNAM, Av. Universidad 3000.

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# *In vitro* import of subunit Atp6 of the ATP synthase into mitochondria of the chlorophycean alga *Polytomella* sp.

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The mitochondrial ATP synthase complex of chlorophycean algae, such as *Chlamydomonas reinhardtii* and *Polytomella parva*, have some subunits similar to those present in other ATP synthase complexes, including mammals, yeast and plants. These subunits are well conserved both in sequence and in structure, but in the case of algae, they are all nuclear encoded. These subunits acquired a presequence (or Mitochondrial Targeting Signal = MTS) which allows them to be imported into mitochondria after being synthesized in the cytosol. In *C. reinhardtii* the Atp6 subunit, also called subunit *a*, has a MTS of 107 residues, while the one of *Polytomella* is 94 residues long. This feature made the chlorophycean Atp6 subunit an exciting possible tool to be used in gene therapy to treat human mitochondrial-Atp6 related diseases. The *C. reinhardtii* Atp6 subunit has been previously used in allotopic expression experiments; the protein was successfully targeted to mitochondria, but it did not assembled into the ATP synthase. Only the assembly pathway from the mitochondrial-encoded yeast Atp6 has been characterized. We think it is important to study which pathway does a cytosol-synthesized Atp6 subunit takes to reach the mitochondrial inner membrane when it is in naturally nuclear-encoded.

We analyzed the Atp6 sequences of several organisms and compared the hydrophobic profiles to obtain clues about the import mechanism. We found differences in the length of MTS in plants and in chlorophycean algae, but the hydrophobicity of the first transmembrane segment is highly similar between all mitochondrial-encoded Atp6 sequences. We point out the significance of the N-terminal length and the hydrophobicity of the sequence in the route of import of the protein. We also carried out *in vitro* experiments to show the import of radiolabeled protein ([$^{35}$S] Met). The open reading frame of Atp6 from a cDNA library, was cloned into the pGEM-T easy vector, and then sub-cloned into the pSP64 vector. We used the TnT Coupled Wheat Germ Extract System to obtain the *in vitro*-synthesized radiolabeled protein. The mitochondria isolation was performed from *Polytomella sp.* cultures and then used to make the *in vitro* import reactions. These experiments are focused to show the maturation of the protein after the import into the mitochondria, as a requirement previous to its assembly into the ATP synthase complex.

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# CatSper, a specific cation channel of sperm is involved in sperm chemotaxis in sea urchin

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In many species including mammals, spermatozoa are guided to the eggs by chemoattractant molecules released by female gametes[1]. The chemoattractant speract is the founding member of the family of sperm activating peptides that modulates sperm motility in marine invertebrates[1,2]. $Ca^{2+}$ plays a key role in sea urchin sperm chemotaxis. CatSper, a sperm specific $Ca^{2+}$ channel, has a fundamental in mammalian fertilization. Males lacking any of the CatSper genes are infertile[3,4]. The aim of this study was to determine the participation of CatSper in sea urchin sperm chemotaxis. The genome of the purple urchin, *Strogylocentrotus purpuratus* contains 6 out of the 7 CatSper genes and the proteins they encode are expressed in sperm from this species. NNC-055-396 and Mibefradil are two CatSper blockers that partially inhibit the $Ca^{2+}$ increases induced by speract[5]. In this work, we document that both CatSper blockers affect the basal motility and chemotactic responses triggered by speract in *Lytechinus pictus* and *S. purpuratus* sperm. Immunohistochemistry and confocal microscopy experiments lead us to conclude that CatSper is located in the flagellum of *S. purpuratus* spermatozoa. Our results support the functional participation of CatSper channels in sea urchin sperm chemotaxis[5].

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# UMAG_02301 gene encoding a transcription factor is involved in the pathogenesis of *Ustilago maydis* in maize

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*Ustilago maydis* is a Basidiomycota fungus, that belongs to the Ustilaginomycotina subphylum. It is the causal agent of the disease known as common smut or “huitlacoche” in corn (*Zea mays*), producing economic losses for millions of dollars world wide. In contrast, it is edible and used in the mexican gastronomy. *U. maydis* has become an attractive model for the study of several biological processes in fungi, including morphogenesis, cell differentiation, and plant pathogenesis. Data from our laboratory demonstrated that under axenic conditions, *U. maydis* is able to infect a number of plant species unrelated to their natural hosts, including the plant model, *Arabidopsis thaliana*. We have proceeded to identify the up-regulated genes during Arabidopsis infection by use of microarrays, in order to identify possible virulence factors. Among them, we identified UMAG_02301 (fold chage=27) gene, that encodes a putative transcription factor. In the present study, we obtained the corresponding mutant using the technique of Double-Joint PCR. The cells were more sensitive to KCl and NaCl stress, but not to $H_2O_2$. The yeast-to-mycelium dimorphic transition was not affected, but the cells were longer than the wild type ones, suggesting a delay in the cell cycle. More important was the observation that the mixture of sexually compatible mutants showed reduced virulence to the natural host (maize) compared to the wild type strains (50%), demonstrating that the gene plays an important role in the fungal virulence.

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# Purification and characterization of digestive trypsin-like and chymotrypsin-like proteases in the larger grain borer *Prostephanus truncatus* (Horn)

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The larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera; Brostichidae), is native to meso-America but it was accidentally introduced into East Africa in the late 1970s, where it has long been recognized as a destructive pest of stored maize and dried cassava (Hodges, 1994). Destructive out- breaks of *P. truncatus* in stored commodities understandably focused the attention of the international research community on elucidating the insect's biology as a pest of stored cereals and, to a lesser extent, stored starchy tubers, in order to develop mechanisms for pest control (Nansen & Meikle, 2002).

Different types of proteases in this insect gut has been reported, but the predominant proteolytic activity of *P. truncatus* comprises serine proteases and are found in the region of the midgut (Preciado-rodríguez *et al.*, 2000). It also presents a pH gradient ranging from 4.2 to 6.2 (Brioschi *et al.*, 2007; Vázquez-Arista *et al.*, 1999).

The partial purification of digestive serin proteases, was done by the precipitation with ammonium sulfate, hydrophobic interaction chromatography and ion exchange chromatography. The fractions showing specific proteolytic activity were concentrated by ultrafiltration and purity of each peak was evaluated by SDS-PAGE electrophoresis and by zymography. Also the partial characterization of the enzymes of interest was determined, including its apparent molecular weight, isoelectric point and their interaction with different protease inhibitors. The digestive origin of the proteases trypsin-like and chymotrypsin-like in the insect pest *P. truncatus* was confirmed by a biochemical comparison between a larval crude extract and dissected intestines. This information will be helpful to achieve a broader understanding of the biological role of proteases in the insect, which would lead to a biotechnological application for the biological control of the larger grain borer.

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# "Easy Expression and Purification of Histidine-tagged Bacteriophage T7 DNA Polymerase in a 1:1 complex with Thioredoxin"

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T7 DNA Polymerase is an enzyme which has the ability to elongate long stretches of DDNA. The processivity of T7 DNA Polymerase is increased when the Thioredoxin of *E. coli* forms a complex with this Polymerase; this binding increases the affinity of the Polymerase specifically to a primer-template by 80 fold. The purification process to obtain this complex is a time consuming and requires four purification steps. To overcome these problems, we have implemented an approach involving a T7 DNA Polymerase with a 9X Histidine Tag with a PPS (PreScission Protease) cleavage site, and a Thioredoxin with a 6X Histidine-MBP (Maltose Binding Protein) Tag. To achieve this construct, we used the Vectors pET19b and pCRI1b respectively. There have been previous studies of the purification of these proteins together, but none of them were able to obtain a 1:1 ratio of the Polymerase-thioredoxin complex. We purified the proteins in separate ways and incubated them together to perform a pull down experiment. We expect to obtain the protein complex Thioredoxin-T7 DNA Polymerase in a 1:1 proportion and in a shorter period than the ones reported nowadays.

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# Physicochemical characterization of monomeric proteins with (β/α)8-barrel topology: looking for unfolding reversibility

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The $(\beta/\alpha)_8$ topology, also known as TIM barrel, is one of the most abundant structural arrangements of proteins in nature. TIM barrels are a versatile scaffold for protein design and engineering that could be used for numerous applications in synthetic biology, diagnostics, therapeutics and imaging. Notwithstanding their importance and abundance, the irreversibility in unfolding transitions has hampered the study of the thermodynamic stability in this protein architecture. Monomeric proteins are scarcely characterized and less prone to irreversible aggregation; therefore, we selected two monomeric TIM barrels for thermodynamic characterization.

Material & methods: We selected two monomeric TIM barrels with biomedical/biotechnological importance: Aldose reductase (AR) from Homo sapiens and 2,5-Diketo-D-gluconic acid reductase A (2,5-DKGRA) from Corynebacterium sp. The synthetized-genes were subcloned into pET28b(+) and overexpressed in Escherichia coli BL21(DE3)pLysS. Proteins were purified using Ni-affinity and anionic exchange chromatography. Spectroscopic properties were determined using Intrinsic-Fluorescence (IF) and Circular Dichroism (CD).

Results: Both proteins were purified to homogeneity. Their far UV-CD native spectra showed minima at 210 and 220 nm, a characteristic feature of β/α proteins. IF λmax (330 nm) indicated that tryptophan residues are buried from the solvent and that these proteins are properly folded. Temperature-induced unfolding was then studied by CD. For both proteins, the native spectra were not recovered after heating the samples to 75°C and cooling them back to 25°C; hence, their thermal unfolding is irreversible. The apparent melting temperatures (Tm) were: 56.5±0.1°C for AR and 40.1±0.1°C for 2,5-DKGRA.

Conclusion: AR and 2,5-DKGRA were purified and partially characterized. Both proteins are catalytically active; spectroscopic studies indicate that proteins are correctly folded. In both cases, temperature-induced unfolding was found to be irreversible. The estimation of conformational stability of monomeric TIM barrels is a very challenging task; nevertheless, we will continue looking for unfolding reversibility of monomeric TIM barrels in order to explore the thermodynamic properties of this protein topology without the contribution of quaternary interactions.

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# Characterization of the hydrophobic subunits of the peripheral arm of the ATP synthase from the colorless alga *Polytomella* sp.

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The mitochondrial $F_1F_O$-ATP synthase of the chlorophycean algae, *Chlamydomonas reinhardtii* and *Polytomella* sp., is a highly stable dimeric complex with a molecular mass of 1,600 kDa. This enzymatic complex is composed of seven classical ATPases polypeptides (*α*, *β*, *γ*, *δ*, *a*, *c* and *OSCP*) in addition to nine subunits with a molecular mass from 7 to 60 kDa, of unknown evolutionary origin and without clear counterparts in databases, which were named *Asa* (Mitochondrial ATP Synthase Associated Protein), and were successively numbered as subunits *Asa1-9*.

We are interested in characterizing the interactions of these subunits, because previous studies in the laboratory support the idea that some of these could constitute the basic components of a peripheral arm (*Asa*1-5 and *Asa*7), others may participate in dimerization enzyme (*Asa*6-9), and/or play a regulatory role (*Asa*?).

As mentioned above, we have described the interactions of the soluble proteins associated to the peripheral arm (*Asa*1, 2, 4 and 7). However, little is known about the subunits that form part of the membrane fraction (*Asa*6-9), as suggested by several studies *in silico* (according to the characteristics predicted by their amino acid sequences). In this work, we propose to approach the study of the interactions of hydrophobic peripheral subunits of ATP synthase arm in order to learn more about the membrane domain of this enzymatic complex.

So far, we have been obtained the recombinant subunits *a*, *c*, and *Asa*6-9 using a bacterial expression system, these heterologous proteins were purified by affinity chromatography and polyclonal antibodies were generated for the subunits *Asa*6-9. Also, the interaction *Asa*6-*Asa*6 was determined qualitatively by co-purification assay and yeast two-hybrid system. Also, the interaction *Asa*9-*Asa*8 was assessed using Far-Western blot and yeast two hybrid system. Likewise, we observed in this *in vivo* approach the interactions of the subunits *a*-*c*, *Asa*6-*a*, *Asa*6-*c*, and *Asa*9-5. A model for the topological disposition of these subunits is proposed.

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# Cloning, optimization, expression and purification of the α-glucosidase *Ruminococcus obeum*, to be used as a molecular target in the discovery of new antidiabetic drugs.

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Glycosidases are molecular targets of drugs, acting at the level of intestinal absorption of glucose, these enzymes catalyze the final step of the digestion process of the carbohydrates, performing the hydrolysis of the glycosidic bond of polysaccharides, oligosaccharides and glycoconjugates. They belong to the families of glycosyl hydrolases (GH) and are essential for degradation of various types of carbohydrates to monosaccharides for absorption and utilization by the organism. The most important enzymes are glycosidases β- and α-, which catalyze the rupture of the carbohydrate glycosidic bond releasing glucose. The α-glucosidase have gained special interest as molecular targets since inhibition of these, has proven to be useful to slow down the absorption of glucose and reduce postprandial glucose levels in blood, and therefore are considered of importance in Therapeutics of Diabetes. Inhibitors of α-glucosidases, act in the intestine inhibiting these enzymes; causing a decrease in the glucose absorption with the consequent disappearance of postprandial hyperglycemia characteristic, of this disease. Acarbose, miglitol, and voglibose are examples and prototype drugs of the α-glucosidase inhibitors. In this work, the expression, purification, kinetics and stability of the α-glucosidase enzyme was characterized of *Ruminococcus. obeum*. Furthermore, it was characterized by means of molecular dynamics, the union of these two enzymes with their main inhibitors, and a good analogy with the experimental data reported for inhibitors was found

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# Effects of Cu(II) and Zn(II) in HgD crystalline over time.

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Cataracts formation is the leading cause of blindness worldwide, affecting 20 millions of persons per year. Currently, the only effective treatment for cataracts is surgical removal, however this procedure is expensive and remains unavailable to most of the people in developing countries. This disease is characterized by the opacification of eye lenses due to
crystallins agreggation. The crystallins are proteins found in the eye lens at high concentrations, and are the responsible for the characteristic lens transparency. The human gamma D (HgD) crystallins is one of most abundant proteins in the lens and it has been linked with cataracts. UV radiation, reactive oxigen species and metals ions are some of the risk factors to the development of cataracts. Specifically, metal ions like Cu(II), Zn(II) and Cd(II) have been found at higher concentration in cataract lenses. In this study, we have characterize the effect of Cu(II) and Zn(II) in the HgD crystallin aggregation over time by different spectroscopic techniques. Our results suggest that both metal ions accelerate the HgD crystallins aggregation without inducing protein unfolding.

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# Effect of human alfa crystallin peptides in the human γD-crystallin aggregation

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Human eye lens consist of differentiated cells in layers, with high concentrations of crystallin proteins. The main function of these proteins is to confer transparency, solubility and stability to the lens. There are three types of crystallins, the α, β and λ.

Human γD crystallin (HgD) is one of the most abundant proteins of the human eye lens. It has 173 residues, is monomeric and is highly soluble, even at high concentrations  Although the intrinsic stability of HgG, several environmental, such as aging, UV radiation, metal ions, etc, and innate factors lead to the insolubility of the proteins and their aggregation, leading to lens opacity, also known as cataracts, which is the first cause of blindness in the world.

Recently, it was reported that cataracts contain, not only aggregated crystallins, but alfa crystallin proteolytic fragments. Some of these fragments have been observed to accelerate or inhibit the aggregation of beta and gamma crystallins. In this project we are evaluating the effect of four of these alfa crystallin peptides in the HgD aggregation by turbidimetry, fluorescence, dynamic light scattering and nuclear magnetic resonance, in order to undesrtand the role of these fragments in the development of the desease.

**Keywords:** Human γD-crystallin, cataracts, aggregation, alfa crystalline, peptides.

# SPECTROSCOPIC STUDY OF Cu(II) BINDING TO LIGHT CHAIN 6aJL2-R24G AND ITS EFFECT ON AMYLOID FIBER FORMATION

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Light chain amyloidosis or primary amyloidosis is one of the most common systemic amyloidosis, characterized by the deposition of immunoglobulin light variable domain as insoluble amyloid fibers in vital organs and tissues, leading to death of patients in a few months. Germline ʎ VI are closely related with this disease and has been reported that 25 % of proteins encoded by this germline have a mutation at position 24 where Arg is replaced by a Gly (R24G). In vitro studies have shown that this mutation reduce the stability and increase the propensity to form amyloid fibrils. In different systems, like Alzheimer, Parkinson and Prion, the role of metal ions have proved to be relevant. For this reason, we studied the role of Cu(II) in the amyloid fibrillization of the recombinant protein 6aJL2-R24G by different spectroscopic techniques. It was observed that Cu(II) accelerate the 6aJL2-R24G fibrillization by binding, with micro molar affinities, in the His99 and His8. These results provide important information in the molecular characterization of amyloidogenic diseases.

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# Kinetic characterization of paralogous enzymes in *Saccharomyces cerevisiae* Bat1 and Bat2: branched aminoacid aminotransferases

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Comparative studies suggest large DNA segment duplication is an evolutionary continous process in the organisms and contributes into genomic plasticity to be the main source for biological innovation. *BAT1* and *BAT2* paralogous genes comefrom the whole genome duplication in *S. cerevisiae* and code for transaminases which participate in branched aminoacids metabolism. Bat1 and Bat 2 share 77% of identity. Bat1 is a 393 aminoacid and 43 KDa protein with an isoelectric point of 8.7; on N-terminal region has signal peptide for mithocondrial destination. Meanwhile Bat2 is a citosolic protein with 376 aa, 41 KDa and a PI of 6.9. These enzymes are involved in valine, leucine and isoleucine metabolism. Previous results in our laboratory showed *BAT1* and *BAT2* expression profile is dependent of the carbon source: *BAT1* is mainly expressed in the beginning of the growth when is glucose in the medium, otherwise *BAT2* is expressed in the end when ethanol is predominant as a carbon source. In this study we characterized Bat1 and Bat2 proteins using molecular exclusion chromatography, we observed these enzymes are dimeric, however, although there is a high affinity among them, these ones present different kinetic behaviors. Bat1 in presence of αKIV has a $V_{max}$= 9.07 and a $K_m$= 64.5 meanwhile $V_{max}$ in Bat2 is 18.6 and $K_m$=85.5. Using intrinsic fluorescence we observed the denaturalization pattern is very similar between these enzymes in native state showing a $\lambda_{max}$ = 338 and a score of 360 which is according with the denaturilazed protein.

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# Purification and characterization of Alt1 and Alt2 of *Saccharomyces cerevisiae*: Study of structural divergence

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*ALT1* and *ALT2* are *S. cerevisiae* paralogous genes, which originated from a Whole Genome Duplication (WGD). Alt1 and Alt2 proteins have a 65% identical amino acid sequence. Reconstruction of the evolutionary history of Alt proteins in the hemiascomycetes group uncovered the fact that in the *Saccharomyces sensu stricto* yeasts Alt1 and Alt2 are distributed in two clades, indicating that there was a diversification process, which resulted in the formation of two distinct clades. These proteins have diverged in subcellular localization, since Alt1 is located in the mitochondria, while Alt2 is cytosolic (this work). The *ALT1* and *ALT2* expression profiles have also diverged, since *ALT1* has a catabolic expression profile: in the presence of alanine *ALT1* expression is induced, while *ALT2* expression is repressed in the presence of alanine, indicating a biosynthetic profile (Marquez *et al* unpublished results).

Previous works from our laboratory demonstrate Alt1 has alanine aminotransferase activity, and it constitutes the sole catabolic pathway for alanine utilization, and the major pathway for alanine biosynthesis. Contrastingly, the function for Alt2 remains unknown, and it does not show alanine aminotransferase activity. The aim for this project is purify and characterize Alt1 and Alt2 to perform a structural comparison in order to determine whish structural differences made the lack of alanine aminotransferase activity. So far we purified and made the enzymatic characterization for Alt1 and Alt2.

Analysis of their secondary structure through far UV spectra with Circular Dichroism, their tertiary structure through Intrinsic Fluorescence and Near UV spectra of Circular Dichroism, and their quaternary structure by Size Exclusion Chromatography (SEC) and Dynamic Light Scattering (DLS) showed that both proteins have similar secondary, tertiary and quaternary structure.

Stability analysis of Alt1 and Alt2, through unfolding curves with guanidine (follow by Intrinsic Fluorescence) and thermal denaturalization (Follow by Circular Dichroism at 222 nm) show that both proteins have similar stability.

# Identification of an-arginine endoplasmic reticulum (ER) retention motif and induction of oocyte maturation by a G-protein activated potassium channel

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GIRK5 is an endogenous potassium channel in *Xenopus laevis* oocytes. Dephosphorylation of a tyrosine (Y16) at the N-term of GIRK5, determines its surface and functional expression[1]. Previously we identified a di-lysine ($Y^{16}$PiEXXXLI)[2] ER motif in this channel. However, GIRK5 presents another potentially di-arginine ($K^{13}R^{14}$) ER retention motif.
Furthermore, previous observations from our laboratory confirmed the appearance of the germinal vesicle breakdown, in the absence of progesterone, after the surface expression of GIRK5 in the oocytes.
In this work we aim to explore if $K^{13}R^{14}$ corresponds to an ER retention motif and also, the physiological role of GIRK5 in the oocytes. We performed an alanine scanning mutagenesis of $K^{13}R^{14}$. K13A mutant did not produce functional channels. In contrast, R14A displayed inwardly rectifying currents. To confirm that the sequence RXY corresponds to an ER retention motif, this sequence was introduced into the C-terminal of a voltage-dependent non-selective cationic channel (HCN2-RXY). This chimera reduced drastically the sodium inwardly currents by HCN2.
RNA interference provides a fast and easy strategy to depleting mRNA by introducing double-stranded RNA homologous (siRNA) to a particular cellular mRNA, leading to its sequence specific removing [(3)]. To evaluate if GIRK5 channel participates in oocyte maturation, a double-stranded GIRK5-specific siRNA oligo was injected into *Xenopus* oocytes. The effects were compared against those of a scrambled control siRNA oligo. Injection of a GIRK5 siRNA oligo reduced GIRK5 channel expression retarding the process of maturation, whereas a control siRNA oligo had no effect.
In conclusion, KRXY is an “arginine-phospho-tyrosine” ER motif and functional expression of GIRK5 stimulates oocytes maturation.

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# Regulation of the hyperpolarization-activated cyclic nucleotide-gated HCN channels by the serum and glucocorticoid-inducible kinase 1 (SGK1)

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) cationic currents are responsable for generating spontaneous pacemaker potentials in the heart and in the central nervous system. Four members of the HCN family (HCN1–4), have been identified and extensively characterized in heterologous systems. HCN channels are also found in non-excitable tissues in mammals where their physiological role has been elusive.

Recently, our laboratory demonstrated that the HCN family participates in the acid-base homeostasis mediated by the kidney.[1,2] In particular, HCN2 is localized in the acid-secreting intercalated cells in the rat kidney and transports ammonium ($NH_4^+$) and sodium ($Na^+$) ions in these cells[1].

$Na^+$ and $K^+$ balance are achieved in distal segments of the nephron under hormonal control (ASDN) by aldosterone, vasopressin, angiotensin II and insulin[3]. The mammalian ASDN plays an important role in $K^+$ handling by the nephron. $K^+$ is transported into the cell across the basolateral $Na^+$–$K^+$-ATPase pump and, in basal conditions, is secreted into the urinary space by passive diffusion through apical secretory $K^+$ channels[3]. The expression of these channels is up-regulated by a high $K^+$ intake (HK) whereas is down-regulated by a low $K^+$ diet (KD)[4].

Previously, we evaluated the effect of HK,KD and metabolic acidosis on the protein abundance of HCN2. We found by immunofluorescence assays that HCN2 was up-regulated only by HK in the distal nephron (unpublished). Aldosterone plays a pivotal role in NaCl absorption and K+ urine excretion, following a HK diet. Kinase SGK1 mediates aldosterone signaling by the activation of the epithelial sodium channel (ENaC) and ROMK channel in the late distal convoluted tubule DCT2, connecting tubule (CNT) and CCD, and of the $Na^+Cl^-$ cotransporter (NCC) in the early distal convoluted tubule (DCT1)[5].

HCN2 has, at the carboxyl terminus, a consensus sequence RXRXX (S/T) for SGK1. The purpose of this work was to study the effect of SGK1 on the activity of HCN2. We performed functional expression assays in *Xenopus laevis* oocytes by applying the two electrode-voltage clamp technique. We studied the human potassium Kv1.3 channel as a control. Our results reveal that SGK1 kinase does not regulate HCN2. Therefore, other kinases were explored to understand the regulation of HCN2 in the distal nephron (CNT and CCD) by a HK diet.

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# Cloning, Expression and Kinetics of the Enzyme Amino Aldehyde Dehydrogenase PA5312 of *Pseudomonas aeruginosa*

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Aminoaldehyde dehydrogenases (AMADHs) catalyze the irreversible, $NAD(P)^+$-dependent oxidation of aldehydes that have an ω-amino primary or quaternary group. Phylogenetically, these enzymes are grouped in five families: ALDH9, ALDH10, ALDH25, ALDH26 and ALDH27. The opportunistic human pathogen *Pseudomonas aeruginosa* possesses one ALDH9 enzyme, involved in coline catabolism and synthesis of the osmoprotectant glycine betaine, and three ALDH27 enzymes, one of which is coded by the gene PA5312. By genomic analysis it has been found that PA5312 is involved in catabolism of arginine and the polyamine putrescine, but the enzyme has not been kinetically or structurally characterized yet. We report here the cloning of the PA5312 gene and the over-expression of the enzyme in *Escherichia coli* cells transformed with the plasmid pET28b(+)-*kauB*, which contains this gene. The enzyme was purified to homogeneity and by size exclusion chromatography was found to be tetrameric. The kinetics with different aminoaldehydes showed that it cannot use betaine aldehyde as substrate, and that ω-aminobutyraldehyde is a better substrate than ω-aminopropionaldehyde. Also, it has a clear preference of $NAD^+$ over $NADP^+$, in agreement with its catabolic role. Using homology modeling and docking simulations, we explored the structural bases of the aldehyde substrate and coenzyme specificity of this enzyme and found possible critical amino acid residues.

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# Cisplatin induced damage in LLC-PK1 cells: cytoprotective effect of Morin without interfering with cisplatin toxicity on HTB-4 cells.

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Antineoplastic drugs induce side effects in patients receiving chemotherapy. Cisplatin (cis-diamminedichloridoplatinum(II)) is a chemotherapeutic drug broadly used in the solid tumors treatment. However, this drug can induce several adverse effects. Nephrotoxicity is the most important among then. About 30 % of cisplatin-treated patient developed acute kidney injury. Toxicity of cisplatin has been associated with oxidative stress, inflammation and apoptosis. Until now, there is not a completely effective treatment to avoid cisplatin nephrotoxicity. On the other hand, flavonoids, natural compounds founded in several plants, have anti-oxidants properties. Morin, a yellow chemical compound isolated from *Maclura pomifera*, has been associated with anti-oxidants, anti-apoptotic and anti-inflammatory effects. The cytoprotective effect of morin has been demonstrated in both *in vitro* and *in vivo* models. Nevertheless, morin decrease viability in some tumoral cells. Taking account this elements, morin could attenuate cisplatin-induced kidney injury without interfered with antineoplastic activity of cisplatin. The main objective of this study was defined and characterizes the cytoprotective effect of morin against cisplatin damage in the LLC-PK1, a proximal tubular cell line derived from normal pig kidney. Morin (200 µM) attenuates cisplatin damage on LLC-PK1 cell, decrease viability of urinary bladder derived from transitional human cell carcinoma (HTB-4) and do not affect the cisplatin toxicity on these cells. Morin increases heme oxygenase expression at 11 h, which is a possible explanation for cytoprotective effect observed.

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# Has the heterologous expression of *Debaryomyces hansenii* catalase T any effect in *Saccharomyces cerevisiae* chronological life span*?*

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*Debaryomyces hansenii* has been described as an euryhaline, cryotolerant, lipolytic and lipogenic yeast; for this reason it is considered as a non conventional yeast. It was first isolated from the sea, so it was considered as a marine yeast; however, it is able to grow on decaying wood, ice cream, sausages, cured meats, cheese, etc. Furthermore, it can use a variety of non-fermentable carbon sources like methanol, ethanol, and glycerol. *D. hansenii* tolerates high concentrations of hydrogen peroxide: it is able to survive to a 30 mM $H_2O_2$ shock when grown in rich media and during the exponential growth phase without significantly compromising the cell viability, while *S. cerevisiae* is severely affected with a 15 mM shock.

*D. hansenii* has two genes encoding catalases, *Dh*CTT and *Dh*CTA; by previous studies we know that catalase specific activity of *D. hansenii* is 24 times higher when compared to *S. cerevisiae* activity in rich medium (YPD). There is evidence that *S. cerevisiae* catalase T overexpression under caloric restriction increases significantly the chronological life span of this yeast. Aging is a multifactorial process; one of these factors is the reactive oxygen species (ROS) unbalance. The theory of aging by ROS proposes that this molecules damage biomolecules, and this damage accumulates over time, causing cell malfunction, leading to aging and related diseases.

In previous experiments we found that an acatalasemic strain of *S. cerevisiae* carrying the monocopy plasmid pRS316 with *Dh*CTT gene, exhibited an increased growth rate in YPD; similarly, the catalase specific activity of the transformant strain is about 10 times higher than that of wild type strain. For these reasons, the heterologous expression of *D. hansenii* catalase T in *S. cerevisiae* could increase the chronological life span of its host; however, since the expression of *D. hansenii* catalase T is not stable in pRS316 we decided to integrate the gene into the *S. cerevisiae* genome at the TRP1 locus by homologous recombination.

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# Measurement of Markers Lipid Peroxidation (MDA and 4-HDA) In Blood Serum In Response To Stressful Conditions in students.

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Stress is one of the main factors that affects students at various levels, including academic performance. In recent research, it has been shown that physiological stress generates brain damage in some areas due to oxidative stress that leads changes on mood and mental disorders, like depression and anxiety. The aim of this study was evaluate markers lipid peroxidation (MDA and 4-HDA) markers in blood serum in response to stressful conditions in students, 84 subjects (56 females and 28 males) students from Facultad de Ciencias Químicas BUAP were divided in two groups to conduct standardized tests in order to evaluate levels of depression and anxiety, and by applying an academic knowledge test. Blood Serum of subjects were obtained before, during and after the test, and analyzing the production of MDA and 4-HDA. Results showed that men have slight levels of anxiety and depression, while in women have a moderate/severe anxiety and moderate levels of depression in Halminton scale (10% of severe depression, 3% of very severe depression women). In general levels of MDA and 4-HDA for sexual dimorphism was negative. Levels of MDA for Group 1 presented significant differences ($p<0.05$) during the stressful event compared with the results a week after the test, 4-HDA levels showed no significant differences ($p>0.05$). To find out if lipid peroxidation was produced by the stressful event a second analysis was performed in three stages: before, during and after the exam. MDA and 4HDA levels were elevated when students applied the test. In conclusion, it is possible to say the psychological stress can lead to produce a biological stress in students, caused mainly by academic tests that have an impact on their mood.

# Evaluation of Tumour Necrosis Factor alpha (TNF-alpha) and Nitric Oxide gene expression in liver's rats fed with *Spirulina platensis* and subjeted to endotoxic shock.

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Knowledge area: Reactive Oxygen Species

In the present study, we examined the role of nitric oxide (NO) in early-response cytokine production by using a rat model of endotoxic shock in liver. We used male Wistar rats, which were divided into 2 groups of 4 sub-groups each one: control, Spirulina (Arthrospira) platensis, LPS and LPS+Spirulina (Arthrospira) platensis; a dose of Spirulina (Arthrospira) platensis was used at 180mg/kg/p.o. once a day for 8 days. The animals were sacrificed and livers were collected for analysis of TNF alpha and NO gene expression using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA for normalization. The expression of (iNOS) mRNA was increased in group III. Moreover, in group IV, plasma TNF-alpha levels at 4 h of reperfusion and IL-1beta levels at 90 min and 4 h of reperfusion were significantly increased compared with group III. No statistically significant changes were observed between groups I and II in plasma ALT activity, plasma NO levels, circulating leukocyte counts, superoxide generation in the ischemic lobes of liver, and plasma TNF-a and IL-1beta concentrations. The observed enhancement of I/R injury by L-NAME is consistent with the hypothesis that endogenous NO down-regulates TNF-alpha and IL1beta generation, thereby decreasing HI/R injury.

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# Revealing the role of selective mitochondrial autophagy in yeast longevity

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Aging is a multifactorial process caused by accumulation of damage in different cellular components. One of the most important actors that promote such damage to molecules and cells is the mitochondria. It is known that mitochondria has two faces: a beneficial side as the main cellular producer of energy, which is essential for cellular survivorship and, on the other hand, the mitochondrial respiratory chain is the major cellular producer of ROS, one of the main generators of cellular damage and oxidative stress. In such antagonistic interplay between energy and ROS production by mitochondria, the selective recycling of this organelle by mitophagy (selective autophagy of mitochondria) is known to be a key player. However, the mechanistic connection of longevity and mitophagy are poorly understood. Here, we focus in molecular mechanisms and signaling process involved in selective autophagy of mitochondria and their specific role in yeast longevity. To this end, we have generated a dual biosensor to measure the selective mitochondria degradation by autophagy of over ~300 long-lived single-knockout yeast strains, which we monitor by high-throughput flow cytometry. We will present the of key genetic players with specific role in mitophagy and novel molecular mechanisms involved in selective mitochondrial autophagy and yeast aging.

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# Expression analysis of *Debaryomyces hansenii* catalase T in *Saccharomyces cerevisiae* as host.

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To overcome oxidative stress, cells have developed specific responses that implicate different levels of action from the activation of antioxidant molecules and enzymes, like catalases and superoxide dismutases, to the triggering of transcriptional programs in order to get adapted to higher ROS concentrations. These transcriptional programs include the up regulation of several genes, including the ones that code for catalases.

The yeast *Saccharomyces cerevisiae* has two catalase-encoding genes. CTA1 encodes catalase A, which is localized in the peroxisomes and is involved in the degradation of the ROS generated during fatty acid catabolism. CTT1 encodes catalase T, which is a cytoplasmic enzyme, that contends with the $H_2O_2$ that reaches the cytoplasm.

The eurihyaline yeast *Debaryomyces hansenii* has also two catalase-encoding genes (*Dh*CTA and *Dh*CTT), which have a carbon source dependent regulation. In this yeast, the presence of NaCl decreases catalase activity, whereas in *S. cerevisiae*, there is a catalase activity increment when NaCl is added. *D. hansenii* has an inherent resistance to $H_2O_2$, which could be attributed to the fact that it has a basal catalase activity several-fold higher than that observed in *Saccharomyces cerevisiae* under the same culture conditions.

We have cloned both catalase-encoding genes from *D. hansenii (Dh*CTA and *Dh*CTT), including their promoter region, in pRS316 plasmid, and transformed them in an acatalasemic strain of *S. cerevisiae (BY4741 ctaΔ/cttΔ),* successfully reestablishing in both cases catalase activity. In particular, *Dh*CTT transformed strain showed a regulation as *S. cerevisiae* but with catalase activity with *D. hansenii* magnitudes, *i.e.* NaCl increases catalase activity reaching much higher levels. Furthermore, it allows its host yeast to reach a larger biomass ($OD_{600nm}$) and to bear up against higher concentrations of $H_2O_2$, compared to *S. cerevisiae* wild type behavior.

To elucidate the regulation that *Dh*CTT gene follows in *S. cerevisiae,* the role that its endogenous promoter plays in catalase expression, and the reestablishment of catalase activity in the acatalasemic strain, using *S. cerevisiae* S288C as reference, we constructed four plasmids containing catalase T encoding-genes: one with *Dh*CTT, one with *ScCTT1* and two reciprocal chimeras (the former ORFs with swapped promoters *Dh/Sc*CTT1 and *Sc/Dh*CTT). Growth curves, specific catalase activity, and viability after a $H_2O_2$ shock -in a gradient from 0 to 30 mM- are being tested in different carbon sources and salt concentrations (YPD, YPD-0.6M NaCl, YPEtOH, and YPEtOH-0.6M NaCl).

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# Induction of Class III peroxidase secretion from maize scutellum in early postgermination in relation with the season of the year.

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Maize production is one of the most important worldwide. Maize kernels are used for human and cattle nutrition, and for industrial purposes. Chalqueño maize is sow in the Antiplano for tortillas production. During germination, the embryo uses the nutrients stored in the scutellum, and in the early postgermination, as nutritional requirements increase, endosperm reserves must be mobilized; for this, the fibrous layer that isolates the embryo from the endosperm, most be modified. During this process the epidermis from the scutellum release Class III peroxidase (POD) to the apoplast, this enzyme participates in fibrous layer permeabilization that allows a free communication among embryo and endosperm (Corona-Carrillo et al., 2014). The aim of this work is to determinate the factors that induces POD secretion.

Dry mature grains were stored at 40% of relative humidity and 7°C. In summer-winter or winter-spring seasons, grains were collected and the embryos were obtained and germinated in sterility at 25°C for 24 h. After this, two embryos were placed in a well of a multiwall plate, and incubated with 1 ml of: $H_2O$, or $CaCl_2$ 1 mM, or $H_2PO_4^-$ 10 mM; or orthovanadate 5 mM, or a mixture of $H_2PO_4^-$ + $CaCl_2$, or a mixture of orthovanadate+ $CaCl_2$, all of them at pH 5.5 and 6.8. During incubation only the scutellum, not the embryonic axis, were in touch with the medium. After 1 h of incubation at 25°C, the embryos were discarded and the medium was tested for POD activity using guaiacol + $H_2O_2$.

POD activity secreted to the $H_2O$ medium was basal and similar in both pH treatments. Addition of $Ca^{2+}$ to the medium induced a small but not statistical significate change in POD activity at both pH being slightly higher at pH 5.5. Addition of $H_2PO_4^-$ produce a secretion of 7.5 or 2.23 times at pH 5.5 or 6.8, that is why $H_2PO_4^-$ with $H^+$ is the inductor of POD secretion. This effect increased 8.3 times if $CaCl_2$ was added to $H_2PO_4^-$ at pH 5.5; this implies that POD secretion is induced with phosphates at acid pH and this is increased in presence of $Ca^{2+}$.

Embryos from grains stored to the end of winter-spring season shown the same activities, but responses incremented 8.8 times at pH 5.5 with $H_2PO_4^-$ and 11.7 times higher with respect to the basal in the $H_2PO_4^-$+ $Ca^{2+}$ treatment. This implies that stored grains develops a different gradual response, due to the degree of quiescence that must be related to the season of the year.

*Reference: Corona-Carrillo J.I., Flores-Ponce M., Chávez-Nájera G., Díaz-Pontones D.M. (2014). Peroxidase activity in scutella of maize in association with anatomical changes during germination and grain storage. Springer Plus 3: 399.*

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# Relationship between enzymatic and non-enzymatic antioxidants with muscle mass loss in the perimenopausal period

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**Introduction:** Menopause is the permanent cessation of menstruation due to loss of ovarian follicular function that produces an abrupt drop in estrogen levels and the classical signs and symptoms, also body and biochemical changes as muscle mass loss and oxidative stress. The human body have an antioxidant system that counteract the pro-oxidant agent presence, for example the superoxide dismutase enzyme (SOD), glutathione peroxidase (GPx) and catalase, which avoid the damage of tissue. Also in recent years, the antioxidant capacity of non-enzymatic compounds has been investigated such as the uric acid (UA). The UA acts as a free radical scavenger and as a chelator of transitional metal ions which lead to a poorly reactive substances formation. In addition, it's has been related to inflammatory process and the production of radical-oxygen species like superoxide anion, however there is few information about the relation of this antioxidant systems with the muscle mass loss in humans, particularly related to the aging process in women.
**Objective:** To establish the relationship between the enzymatic and non-enzymatic antioxidants with the muscle mass in perimenopause women.
**Methods:** We carried out a cross-sectional study with 93 perimenopausal women (41 to 60 years old). Blood samples were collected after 8-hour fasting period by venipuncture, we measured the activity of SOD, GPx and plasma total antioxidant status (TAS) by commercial kits (Randox Laboratories); the UA and albumin levels were determinated in serum using a Cobas© C111 analyzer and we calculated the antioxidant gap (GAP). The muscle mass was determinated by bioimpedance analysis. Finally, it was calculated the muscle mass index (MMI), skeletal muscle mass (SMM) and free fat mass (FFM).
**Results y discussion:** We found that the SMM was low on postmenopausal women than premenopausal women (17.6±2.3 vs. 18.8±2.1 kg, p= 0.01). The results also show a positive correlation between the MMI and AU (r=0.219, p=0.031), a positive correlation between FFM and UA (r=0.248, p=0.014) and a negative correlation between FFM and SOD (r=-0.200, p=0.05). Apparently the positive correlation of UA and the muscle mass can be an antioxidant response to the oxidative stress generated by the tissular aging. On the contrary, the response of SOD is raised by the production of superoxide anion in the muscular fibers thanks to the aerobic metabolism, ending in the production of hydrogen peroxide. All this pro-oxidant activity affects in a direct way accelerating the tissular damage and diminishing the muscle mass.
**Conclusions:** Our finding suggest that there is an antioxidant response to the loss of muscle mass in the perimenopause.

**Acknowledgements:** This study was supported by grant DGAPA-PAPIIT-UNAM IN224115.

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# Iron mediated-ISC system of oxidative damage and mitochondrial dysfunction in *Saccharomyces cerevisiae*.

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The present study aimed to determine the effect of mutations in the iron sulfur cluster (ISC) genes over oxidative stress control mediated by $Fe^{2+}$ and electron transport chain (ETC) activity in *S. cerevisiae* BY4741 and its *KanMX4* gene mutants in Fe-S assembly system (*ssq1*Δ, *grx5*Δ, *isa1*Δ), iron transport (*atx1*Δ, *mrs4*Δ, *aft1*Δ) and catalytic subunits of the ETC (*sdh2*Δ, *rip1*Δ, *cox11*Δ).

Intracellular $Fe^{2+}$ and reactive oxygen species (ROS) in yeast cultures or cells suspensions were determined using oxidant-sensitive, cell-permeant fluorescent probes ($H_2$DCFDA, DHE and PGFL). Fluorescence was quantified by flow cytometry and confocal microscopy. Mitochondria were isolated and permeabilized for determination of mitochondrial complexes activity (complex II, III, IV) adding specific substrates (DCIP, succinate, cytochrome *c* oxidized and reduced) and inhibitors (antimycin A, KCN).
Our results indicate that intracellular free $Fe^{2+}$ correlate with an increase in ROS generation caused by stressors, due to ISC system dysfunction, which was decreased by the antioxidant addition, ascorbic acid [20mM], under normal growth conditions. To determine if the increment in free $Fe^{2+}$ associated with ROS generation may have originated into mitochondria, we analyze the activity of the complexes II, III and IV from the ETC. Interestingly the activity was impaired or totally abolished in the *ISC* mutants without adding any stressor.

The findings suggests that the free $Fe^{2+}$ content was originated from mitochondrial Fe–S cluster proteins (as one of the main routes) under normal and oxidative stress conditions, affecting ETC activity at complex III level, inducing superoxide generation and increase of intracellular $Fe^{2+}$. In conclusion this study indicates that in *S. cerevisie,* the ISC system is important in iron-homeostasis and ROS stress control, thus affecting the functionality of the respiratory chain.

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# SRX1 encodes a sulfiredoxin required to support the oxidative stress in *Candida glabrata*

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Living organisms generate reactive species as a result of its normal metabolism; in order to control, avoid and repair the damage to biomolecules there are enzymatic and non-enzymatic systems. Owing to the reactivity of their thiol groups, some protein cysteine residues are highly sensitive to oxidation by these reactive molecules, which may perturb cellular homeostasis. Thiol reduction is controlled mainly by the thioredoxin (Trx)/peroxiredoxin (Prx) and glutathione (GSH) systems, which respond to stress situations to regulate redox homeostasis. The opportunistic pathogen *Candida glabrata* is highly resistant to oxidative stress produced by different oxidative agents in vitro, and moreover, it supports the macrophages attack preventing the phagolysosome maturation. From data obtained in our laboratory, we know that the sole catalase, Cta1, is responsible of the high resistance to $H_2O_2$ of C. glabrata; furthermore, superoxide dismutases, Sod1 and Sod2, protect to *C. glabrata* of agents that produce superoxide ion. Additionally, *C. glabrata* cells lacking glutathione are sensitive to oxidative stress and have a reduced late chronological life span. However, individually, none of these systems have a crucial effect in mice colonization assays. In this work, we have characterized the role of sulfiredoxin, CgSrx1, in the oxidative stress response in *C. glabrata*. Sulfiredoxin contributes to oxidative stress resistance by reducing cysteine-sulfinic acid groups in the peroxiredoxins, which is formed upon exposure to oxidants. We constructed *srx1*Δ single mutant and *cta1*Δ*srx1*Δ, *gsh2*Δ*srx1*Δ, *srx1*Δtrr2Δ double mutants. The *srx1*Δ mutant grows like wt strain in YPD and YNB media, and only *cta1*Δ*srx1*Δ and *gsh2*Δ*srx1*Δ double mutant show slow growth in both media. Additionally, *cta1*Δ*srx1*Δ is unable to use glycerol, ethanol or lactate as a carbon source. In acute $H_2O_2$ sensitivity assay we found that *srx1*Δ mutant is resistant like wt strain but *cta1*Δ*srx1*Δ and *gsh2*Δ*srx1*Δ double mutant are more sensitive to $H_2O_2$ stress than *cta1*Δ and *gsh2*Δ single mutants. Moreover, Srx1 is not essential for the adaptation response to $H_2O_2$. In chronic sensitivity assays we found that *srx1*Δ mutant is sensitive to $H_2O_2$ like *cta1*Δ mutant; *cta1*Δ*srx1*Δ and *srx1*Δtrr2Δ mutants are most sensitive to $H_2O_2$. *cta1*Δ*srx1*Δ cells lost viability in stationary phase (SP) when grow in YNB. We made a transcriptional fusion with GFP gene cloned under control of *SRX1* promoter and found that *SRX1* promoter is induced by $H_2O_2$, menadione and cumene hydroperoxide; the induction is controlled by transcriptional factor Yap1. We show that Srx1 is an important enzyme in the response to oxidative stress in *C. glabrata*.

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# Thioredoxin-glutathione reductase is an ancestral enzyme in the Animal Kingdom. An evolutionary proposal about its importance in the antioxidant systems in animals.

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Thioredoxin-glutathione reductase (TGR) is an isoform of the animal thioredoxin reductase with a multifunctional nature. In addition to thioredoxin, the enzyme is also able to reduce the oxidized form of glutathione and catalyze dithiol/disulfide exchange reactions due to the presence of a glutaredoxin-like domain appended at the N-terminal end. In the latter, a dithiol/disulfide redox motif is critical for its multifunctional nature. Originally described in vertebrates, TGR has also been identified and characterized in flatworms (Phylum Platyhelminthes). The presence of the enzyme in these two phylogenetically remote groups pose a challenge to explain their evolutionary origin, as well as its potential presence and importance in other groups within the animal kingdom. To answer this question, a search for TGR-like sequences in the animal genomes deposited in a variety of databases (NCBI, GeneDB, WormDB, etc.) was carried out. Using TGR protein from human (HsTGR) and *Schistosoma mansoni* (SmTGR) as query, a total of 56 sequences, representatives of 20 animal taxa, were found. The sequences were aligned and a phylogenetic analysis was performed. Additionally, from selected sequences the three-dimensional structure of the enzyme was modeled using the crystal of SmTGR as template. From the results obtained, the following conclusions can be put forward: i) a functional TGR is present in basal animal taxa as earliest as Porifera and Cnidaria; ii) a vestigial variant of TGR (i.e. TGR lacking of the dithiol/disulfide redox motif of the glutaredoxin-like domain) was found in nematodes; iii) the group of Panarthropoda is the one with greater complexity, iv) In all of the invertebrates taxa analyzed, as well as in some vertebrates, the glutaredoxin-like domain of TGR is of the dithiol-type; v) In all cases where the three-dimensional structure of the enzyme was modelled, the typical tertiary structure of TGR was found, irrespective of the presence of the dithiol/disulfide redox motif at the glutaredoxin-like domain of the enzyme.

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# Effect of aged garlic extract on the level of reactive oxygen species and phosphorylation of AMPK in muscle tissue of diabetic rats

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Diabetes mellitus is a disease characterized by hyperglycemia resulting from defects in the secretion or action of insulin to regulate glucose levels. Chronic hyperglycemia is associated with long-term damage and dysfunction of various organs, particularly eyes, kidneys and heart. It has been reported that hyperglycemia generates oxidative stress, which is derived from the increase in the production of reactive oxygen species and decrease of endogenous antioxidant defenses. Oxidative stress causes damage to macromolecules, modifying proteins sensitive to oxidative stress and oxidizing lipids, carbohydrates and DNA, causing damage and dysfunction in cell tissues. Pathways involved in glucose regulation such as insulin and AMPK pathway, are altered in a state of oxidative stress. The AMPK pathway may regulate hyperglycemia, but its activation may be diminished due to oxidative stress. Therefore, the use of exogenous antioxidants as aged garlic extract (AGE) may reduce oxidative stress and increases phosphorylation of AMPK. We use a model of diabetic rats (60 mg/kg streptozotocin). The AGE (300 and 200 mg/kg) and metformin (100 mg/kg) was administered orally daily for 28 days. After treatment the muscle tissue was obtained to measure the level of reactive oxygen species by flow cytometry and phosphorylation of AMPK by western blot. In glucose levels only dose of 300 mg/kg in a healthy condition has hypoglycemic effect. Treatment with AGE significantly decreases reactive oxygen species in diabetic rats, both the dose of 200 mg/kg (96.6%) and 300 mg/kg (54.6%) compared to the diabetic group (140.1%). Metformin treatment more AGE, also decreases reactive oxygen species synergistically (64.1%) in diabetic rats. In the other hand, 200mg of AGE in diabetic rats increases significantly the phosphorylating AMPK compared to the diabetic group. In conclusion, the AGE decreases the reactive oxygen species dose dependent, exerting its antioxidant potential and the dose of 200 mg/kg of AGE increases AMPK phosphorylation in an in vivo model of diabetes.

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# Preliminary phytochemical analysis of Guácima (*Guazuma ulmifolia* Lam.) steams, seeds and leafs.

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**Knowledge area:** Reactive Oxygen Species

**Abstract:** In order to improve the knowledge of flora in Mexico, particularly that belonging to the Valley Region in Jalisco state, *Guazuma ulmifolia* Lam was chosen for a chemical composition in–depth study to establish a potential utility in medical or industrial fields. For this analysis, a preliminary phytochemical study of three organs of the plant –steams, seeds and leafs– was made; the purpose was to find the main secondary metabolites associated with biological activities: alkaloids, flavonoids, tannins, saponins, antracenic derivates, steroids, coumarins, cardiotonic glycosides and terpenic lactones. The main compounds were alkaloids, flavonoids, Tannins and polyphenols. The physiological activity of alkaloids on animals, especially on humans renders them important as potential drugs and exhibited a variety of biological activities in controlling recurrent fevers, in ophthalmology, in prevention of motion sickness, in the treatment of high blood pressure, leukemia and anticancer effects. Tannins are known to have antimicrobial properties. Flavonoids, possess antiviral, antifungal and arthritic properties. Triterpenoids are known to possess anti-inflammatory, lipolytic activities. Tannins are known to produce anthelmintic activities. Polyphenols have many health beneficial functions including antimutagenic, anticarcinogenic and anti-aging. These compounds are well known as scavengers, useful in prevention of diseases related with oxidative processes. Finally, the results of this work are very useful as a first approach for knowledge and utility of the vegetal species found in this location. Furthermore, this plant has big agronomical advantages related with the culture establishment, which improves the rational use of natural resources in our country, as a means of regional and national development.

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# Isoliquiritigenin attenuates cisplatin induced proximal tubule cell death

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Cisplatin is an antineoplastic agent widely used in the treatment of solid tumors. However, nephrotoxicity has limited its use. Although it not completely understood the mechanism by which cisplatin induces renal damage has been established that such damage is associated with increased production of reactive oxygen species (ROS) and decreased anti-oxidant defense system. On the other hand isoliquiritigenin (IsoLQ) is a chalcone found in Licorice, one of the most popular medicinal plants in traditional Chinese medicine. IsoLQ has biological activity as antioxidant and anti-inflammatory in models of chemoprevention. In this study, we examined the effect of IsoLQ on cisplatin induced death in LLCPK1 (kidney proximal tubules) cells, we found that IsoLQ attenuated cisplatin decrease cell viability measured by MTT and FDA assay when cells were pretreated with it for 24h. Further IsoLQ (5-25 µM) exacerbates the damage caused by cisplatin in carcinoma cells of bladder (HTB4 o T24), this suggest that IsoLQ does not interfere with the antineoplastic capacity of cisplatin. A possible mechanism that explain this effect citoprotector, is IsoLQ increase of hemo-oxigenase 1 protein levels in a concentration-dependent manner with a 20 fold increase when cells were treated with 25 µM IsoLQ by 24h of pre-treatment. IsoLQ could to induce Nrf2 protein to translocate to the nucleus. Nrf2 is a transcriptor factor that induces genes that participated in antioxidant response, and HO-1 gen is one of these.

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# Computational Study of Chemical Reactivity in Antioxidant Systems.

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On antioxidant defense biochemical mechanisms of glutathione and thioredoxin, the oxidation process of thiols (R-SH) and selenols (R-SeH) is fundamental for reduction of oxidative species such as hydrogen peroxide ($H_2O_2$) and organic hydroperoxides (ROOH). In this work, computer simulations and electronic structure calculations in the framework of the Conceptual Density Functional Theory were done, using a set of small organic compounds like structural models of antioxidant agents, and cysteine and selenocysteine aminoacids. The results show the chemical reactivity of the antioxidant species and allow their comparison. The perspective of this work is to take experience in order to understand the biomolecular function in homologous proteins with antioxidant activity and their evolutionary changes, through theoretical and computational tools.

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# Redox balance in the interaction of *Trichoderma-Arabidopsis*, role of *TaTrx2* thioredoxin

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Reactive oxygen species (ROS) such as superoxide anion ($O_2^-$), hydroxyl radical (OH °) and hydrogen peroxide ($H_2O_2$), are well known to cause damage to the cell when found at high levels. However, internal and controlled oscillations of these molecules are involved in regulating development, differentiation, extracellular signal transduction and in the establishment of biological interactions, particularly as those that present plants and fungi[1], which are of interest. Enzymes such as superoxide dismutase, catalase, NAD(P)H oxidase and thioredoxins are key in modulating ROS levels during these processes[2].

*Trichoderma atroviride* is a filamentous fungi common inhabitant of the rhizosphere, able to associate with plants, generating beneficial effects related to growth and response to biotic and abiotic challenges[3]. Previously, in our working group, Guzman-Guzman identified the gene TaTrx2 of *T. atroviride*, it was differentially expressed in interaction with *A. thaliana.* This gene encodes a protein disulphide isomerase PDI, a member of the family thioredoxin. To analyze the role of this enzyme Guzman-Guzman generated the necessary constructions to obtain overexpressing and mutants strains of this gene, and a translational fusion of *TaTrx2* gene with the coding region of *mCherry* gene, as reporter system, obtaining in each case transformants strains of *T. atroviride*. This work aims to determine the involvement of the TaTrx2 thioredoxin in the balance of ROS during interaction *A. thaliana -T. atroviride*, will be confirmed the mutation and overexpression of the gene and will be determinate TaTrx2 participation in the oxidative stress response of *T. atroviride* and during interaction with the plant. We have confirmed two null mutants showing increased susceptibility compared to the WT strain to the presence of hydrogen peroxide in the culture medium.

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# Functional Nox4 is required for development of progressive motility in spermatozoa of guinea pig

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The spermatozoa acquire the progressive motility in the epididymis during the last stage of the maturation process. Thanks to this motility, the spermatozoa is able to fulfill its only function: to fertilize the oocyte. The processes that allow the spermatozoa motility are still unclear, although it is well known that many cytoskeleton proteins (actin, tubulin and septins) have a fundamental role.

In the spermatozoa, septins have been related to the barrier formation that maintains the cell compartmentalization. In fact, the *annulus*, an structure localized in the middle piece of the spermatozoa and that presumably allows the flagellum movement, is formed principally by septins. Septins are GTP binding proteins that forms hetero-oligomeric complexes and structures of high-order structures like filaments and rings that are related with the formation of intracellular compartments and diffusion barriers.

Somatic cells from mutant Sept4 mice do not show any particular phenotype. Nevertheless, these mice have immobile spermatozoa that lose their annulus and fails in the last step of the maturation process (cytoplasmic drop release). On the other hand, different members from the NADPH oxidases family (NOX) have been described in mammalian spermatozoa; it has been proposed that the production of reactive oxygen species (ROS) from NOX is fundamental for a correct spermatozoa capacitation. In addition, it has been suggested that the interaction between septins and NOX allow the organization of actin and tubulin cytoskeleton during migration of epithelial cells.

The aim of this project is to understand the effect of ROS production on guinea pig spermatozoa capacitation, phosphorylation and their motility development. Using WB, we detected Sept4 and Nox4 in guinea pig spermatozoa. Both, production of ROX and spermatozoa viability in the presence of inhibitors of proteins related with the production of ROS (DPI, NSC23766 and Vas2870), were quantified by flux cytometry. Levels of ROS and progressive motility of the spermatozoa decreased only in the presence of Vas2870, a specific NOX inhibitor. In addition, an increase in the amount of viable spermatozoa was observed with this inhibitor. The interaction between Nox4 and Sept4 during spermatozoa capacitation was evaluated using co-immunoprecipitation again in the presence of Vas2870. No changes were observed in these proteins interaction. This results suggest that development of progressive motility in the guinea pig spermatozoa requires ROS production from Nox4 and that Sept4 interaction whit Nox4 is a process independent of the presence of ROS produced by Nox4.

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# DEND-CUR-(G2)-OH, a new synthetic compound derivate of curcumin, turns off the overexpression of Nrf2 and causes cellular death by production of reactive oxygen species and autophagic activation on C6 glioma cells.

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**INTRODUCTION:** Glioblastoma (GBM) is a malignant brain tumor, characterized by uncontrolled cellular proliferation, necrosis, angiogenesis, resistance to apoptosis and chemo-resistance. Treatment of GBM consists of surgical resection coupled with ionizing radiation and the chemotherapeutic agent temozolomide. However, this treatment only provides patients a 12–14 month survival period post-diagnosis. Curcumin, a derivative of the Asian spice, curcuma, has several biological activities as anti-inflammatory, anti-cancer, antiangiogenic and antioxidant, but both its poor bioavailability and its water insolubility limit its effectiveness as anti-cancer agent. So, the chemical modification of curcumin could be an effective way to obtain analogues with superior properties.
**OBJECTIVE:** Synthesize a new compound derived from curcumin and characterize the cytotoxic activity in C6 glioma cells.
**METHODS:** We synthesized a new derivative of water-soluble curcumin, through the modification of its phenolic groups with polyester dendrons of second generation, and we denominated it DEND-CUR-(G2)-OH, whose molecular weight is about three-fold higher than that of curcumin; so an equimolar ratio of net curcumin was maintained throughout all assays. C6 glioma cells and normal human fibroblasts were treated with different concentrations of curcumin or DEND-CUR-(G2)-OH, and kinetic studies were carried out. Cellular viability was determined using MTT assay. DNA fragmentation was observed by electrophoresis. Fluorescence microscopy was performed in order to analyze cellular uptake and the presence of markers of apoptosis or autophagy, as well as for monitoring the global levels of ROS in real time. Expression levels of proteins, p62, LC3, Beclin1, Nrf2, eIF2-alpha and p-eIF2-alpha, were evaluated by immunoblot, and glutathione levels were assessed by a colorimetric assay.
**RESULTS:** The new compound induces cell death in glioblastoma cells more effectively than curcumin, because, besides being more stable in the intracellular environment, DEND-CUR-(G2)-OH inhibits expression of factor Nrf2 by two different mechanisms. It promotes the re-location of nuclear p62 to autophagosomes in the cytoplasm, and consequently, Keap1 is released into the nucleus, allowing degradation of Nrf2. Moreover, DEND-CUR-(G2)-OH induces dephosphorylation of factor eIF2-alpha, whereby de novo synthesis of Nrf2 is stop. Inhibition of the expression of Nrf2, causes increased formation of reactive species, and saturation of glutathione system; therefore, the pre-incubation of cancer cells with DEND-CUR-(G2)-OH, before an oxidative challenge, increases its cytotoxicity. None of these responses occurred in the presence of curcumin, which induces apoptosis in C6 cells. Furthermore, DEND-CUR-(G2)-OH, is not toxic to normal human cells, in contrast to curcumin. **CONCLUSIONS:** Our results show that constitutive activation of Nrf2 in glioblastoma cells, that promotes survival and chemoresistance, is due both to the deregulation of the p62/Keap1/Nrf2 axis, as the over-activation of p-eIF2α/ATF4/Nrf2 pathway. Hence, the new compound DEND-CUR-(G2)-OH, which turns off the expression of Nrf2, is specifically directed towards these two mechanisms, and therefore, represents a very promising therapeutic alternative for GBM.

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# Effect of *Moringa oleifera* on kinetic parameters of diabetic liver catalase.

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One main target of glycation stress found in diabetes is catalase. A decrease in its activity may comprise the overall antioxidant enzyme defense system, since hydrogen peroxide ($H_2O_2$) is shown to be a potent inhibitor of superoxide dismutase (SOD). Thus catalase inactivation would lead to oxidative damage not only directly through $H_2O_2$ and its derivate, but also indirectly through inhibition of SOD, hence leading to increased levels of superoxide radical. Some studies have been reported on the effects of extracts of *Moringa oleifera* on any antioxidant enzymes including catalase. However, these studies determinate its activity with commercial kits.

Several experimental methods have been described in literature to study the kinetics of the catalytic decomposition of $H_2O_2$. It is interesting to mention that some recommended methods are qualitative in nature, for example, Choinski´s method. For this reason, the aim of this study was to analyze the kinetic parameters of the enzyme by different methods, spectrophotometric, tube activity and oximetry.

The diabetic rats induced with STZ were divided in two groups; Diabetic control and diabetic treated with *M. oleifera* leaves extract for 21 days. As negative control rats treated with citrate buffer were used. Rats were euthanized to isolate different cell fractions from liver by differential centrifugation. The cytosolic fraction was used to measure calatase activity. In our conditions, the spectrophotometric and $O_2$ selective electrode method only perform well at low $H_2O_2$ concentrations (2-40 mM), while Choinski´s method and the Iwase´s assay were more reproducible at considerably high $H_2O_2$ concentration (50-200 mM). However, all methods used do not allow accurate evaluation of the Michaelis-Menten parameters. Although, the results obtained shown clearly that Diabetic and Moringa groups showed a significant decrease in catalase activity compared to control group.

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# Time-dependent and tissue-specific changes in redox state and oxidative stress during fasting

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Food deprivation exerts profound metabolic changes in cells and tissues to preserve overall organismal energy balance. However, the effect of food deprivation on redox status and reactive oxygen species (ROS) metabolism remains largely unclear. Here, we explore whether fasting systemically modifies cellular redox status in distinct metabolically active tissues. We examined oxidative stress parameters and ROS-related enzymes during fasting for one to three days in the liver, heart, and kidney of rats. We found that fasting differentially regulates ROS level, lipid peroxidation, and the expression/activity of antioxidant enzymes in a time-dependent and tissue-specific manner. Our studies suggest that, in order to deal with nutritional stress, organisms activate complex adaptive, tissue-specific responses to balance systemic redox homeostasis.

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# Hormone therapy decreases oxidative stress in postmenopausal women with metabolic syndrome

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**Background:** The beginning of aging in women it is associating with estrogen deficiency which causes a postmenopausal status with changes physical, metabolic, psychologic, biologic and chemical. The erratic production of estrogens caused by a series of endocrinological changes increases the possibility of metabolic syndrome [MS] (30-35%) and oxidative stress [OS] in these women. Previous studies have reported that hormone therapy [HT] provided to postmenopausal women as a treatment for menopausal symptoms have benefic effect, although the effect of HT in women with very high OS, like postmenopausal women with MS it is not clear.
**Objective:** To evaluate the effect of hormone therapy on markers of oxidative stress associated to metabolic syndrome in postmenopausal women after 6 months of treatment.
**Methodology:** A randomized controlled clinical trial was conducted with 112 postmenopausal women (40-60 ages) forming 4 groups: a) 25 without MS with treatment of 0.625 mg/d to conjugated estrogens (E2) plus 5 mg/10d of medroxiprogesterone; b) 32 with MS and the same treatment; c) 31 without MS with placebo; d) 24 with MS and placebo.
The MS was defined according to NCEP-ATPIII criteria with measurements of waist circumference, blood pressure, glucose, HDL and triglycerides. OS was evaluated with the markers: superoxide dismutase [SOD], glutathione peroxidase [GPx], and total antioxidant status with commercial kits (Randox Laboratories), lipoperoxide levels by TBARS method, and calculated antioxidant gap and SOD/GPx ratio. The measurements were made at baseline, 3 and 6 months.
**Results and discussion:** We found that lipoperoxides levels decreases in the MS-HT group 0.352±0.04, 0.295±0.07 y 0.288±0.07 μmol/L, $p<0.0001$; and in the women without MS-HT: 0.336±0.05, 0.279±0.05 y 0.283±0.05 μmol/L, $p<0.0001$, at baseline, 3 and 6 months, respectively. GPx activity increase in the HT groups, MS-HT: 57.4±20.4, 56.3±14.7 y 72.3±25.9 U/g Hb, $p<0.0001$; and without MS-HT: 53.4±14.4, 56.0±18.9, 62.0±11.9 U/g Hb, $p<0.0001$, at baseline, 3 and 6 months, respectively. Also we observed a decrease in the SOD/GPx ratio in the same groups. In placebo groups there was not change. About this, we can observe that the women who received HT have the benefit to decrease lipoperoxides levels and increase GPx activity, causing a synergy to maintain homeostasis in the organism, emphasizing a highest effect in women with MS.
**Conclusions:** Our results suggest that HT administrated during 6 months, decreases OS in postmenopausal women, with a better effect in women with MS.
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# Analysis of the antioxidant activity of the hexanic extract of *Eryngium carlinae*, *in vitro* and in *Saccharomyces cerevisiae* as biological model

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Oxidative stress has been associated with the onset of chronic degenerative diseases such as cancer, cardiovascular and neurodegenerative disorders. Epidemiological studies have shown the use of plants containing antioxidant is beneficial to health. *Eryngium carlinae* commonly known in México as "*Hierba Del Sapo*" in traditional herbal medicine, attributed with diuretic and healing properties. Currently, ingested as daily infusion is use to regulate blood pressure and as a hypolipidemic agent; however. Different assays ("*in vitro"* and "*in vivo*") have been developed to evaluate the antioxidant activity of plant extracts. The identification and quantification of constituents, through gas chromatography–mass spectrometry (GC/MS) analysis. The antioxidant activity was evaluated using "*in vitro*" assays (total antioxidant capacity, DPPH radical scavenging activity, scavenging capacity towards hydrogen peroxide ($H_2O_2$)), and, analyzing the antioxidant effect on biomarkers of oxidative stress and its influence on the viability in cells of *Saccharomyces cerevisiae* treated with the hexanic extract, and exposed to $H_2O_2$. In chromatographic analysis, the majority constituents found in the hexanic extract of E. carlinae were terpenes and sesquiterpenes, (Z)β-Farnesene (38.79 %) β-Pinene (17.53 %), Calamene (13.30 %), α-Farnesene (10.38 %). "*In vitro*" tests, showed a similar reducing capacity of $H_2O_2$, and an approximate effect of anti-lipid peroxidation obtained with ascorbic acid was observed in our results. In *S. cerevisiae*, the results of viability are correlated in the reduction of levels of cellular lipid peroxidation, protein carbonylation and glutathione ratio in accordance with control. In conclusion, the hexanic extract of *E. carlinae* has antioxidant activity "*in vitro*" and in *S. cerevisiae*, reducing the levels of lipid peroxidation, protein carbonylation, and increasing viability, attributable to farnesene and pinene, and the synergism between the constituents of the extract.

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# Antioxidant activity of bioactive compounds present in the organic waste of the insect *Ulomoides dermestoides*

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Knowledge area**:** Species Reactive Oxygen.

**Abstract**

Mexico has an impressive variety of natural resources that need to be studied. Among those resources insects and their byproducts are fully appreciated at some Mexico´s regions due to their properties. Scientific reports have shown that health-eating habits coupled with a good lifestyle are the key for diminishing ailments and promoting quality life. Some foods or ingredients (bioactive) have functional properties, meaning that along with their basic nutritional functions they trigger a benefic effect on human health.

Nowadays, there are some reports about Chinese weevils (*Ulomoides dermestoides*) consumption, a species that recently has become popular, since its benefic effects have been attributed to human health through coleotherapy, which consist of eating alive beetles to achieve the healing of some diseases. There are few studies about the consumption of these beetles and the benefits provided to health, but there are even fewer that talk about benefits associated with the consumption of beetle's waste.

Due to the research that has been done in functional foods that are only oriented to conventional foods, that´s why this project is focused on the study and analysis of non-conventional foods like the organic waste from the insect *U. dermestoides*. The antioxidant activity has been analyzed by various chromogenic compounds (ABTS, DPPH and FRAP) which are used to determine the ability of phenolic compounds contained in the Chinese weevil`s organic waste to capture free radicals, thereby operating against the harmful effects of the oxidation processes involving reactive oxygen species (ROS).

The applied methods for the analysis of the organic waste were ABTS, DPPH and FRAP, these three methods have an excellent stability in certain conditions, although they show several differences too. This study provides the information, with the purpose of examine and scientifically prove the benefits and popularity that this insect has obtained from many cultures of the world as a Nutraceutical resource.

# Resveratrol effect in cardiomyocytes during aging process in rats

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Nowadays scientific and technological progress has increased life expectancies, this means an increasing number of elderly people. The study of theories of aging aiming to offer a better quality of life during this process has led to new tasks for medical sciences. Cardiovascular risks boost along with aging, this conditions are predominant on population 55 years and up. The oxidative stress produced by free radicals makes up a portion of coronary risk and aging process. The objective of this work is to study new ways to combat oxidative stress through the use of the antioxidant resveratrol. Three months old male rats of the Wistar strain were randomly conformed in 4 groups: target (without treatment), positive control (which was administer with vitamin E), negative control (which was administer with alcohol to ensure that the vehicle does not interferes with the results) and resveratrol (10 mg/kg/day o.r.), the rats were sacrificed within 2, 4, 6 and 8 months of treatment, the hearts were gathered and homogenized with PBS. The supernatant was evaluated for oxidative stress markers, which comprise nitric oxide, MDA + 4-HAD and catalase and superoxide dismutase activity. The results showed that resveratrol does have an important antioxidant activity, since the levels of oxidative stress markers were drastically reduced in regard to the target group and it is efficiency is practically similar to the vitamin E. Regarding the activity of catalase and superoxide dismutase enzymes, it was found that there were no significant differences between the target group and the resveratrol group cardiomyocytes, thus, it is deduced that resveratrol has no influence at all on these enzymes activities. Based on the obtained results, it is concluded that resveratrol features an important antioxidant effect on cardiomyocytes during the aging process on rats, and this effect does not relate with the catalase and the superoxide dismutase enzymes activity.

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# Industrial interest exoenzymes production by acidophilic bacteria isolated from oxidized mine tailings

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**Keywords:** bioprospecting, hydrolases, heavy metals

**Introduction:** Acidophilic bacteria are characterized by inhabiting environments with pH <5 and their ability to biotransform sulfur and resistance to metals. These microorganisms not only play an ecological role but also have economic significance due to they produce extracellular enzymes with high activity and low pH stability such as amylases, proteases, lipases, esterases, xylanases etc., These enzymes hace application in industry food, textile and bioremediation (Sharma *et al.,* 2012). Several works report a high abundance and diversity of acidophilic bacteria in mine tailings and acid mine drainage, Therefore, the aim of this study was to detect the production of industrial interest enzymes by acidophilic bacteria isolated from oxidized mine tailings in Taxco, Gro. **Methods:** 15 samples of acidic mine tailings were collected and determined the concentration of sulfates, total and soluble metals by Inductively coupled plasma atomic emission spectroscopy (ICP-AES). The bacteria were isolated in minimal medium supplemented with acid extracts obtained from the samples. Strains were classified by ERIC-PCR and subsequently identified by amplification and sequencing of 16s rDNA gene. The analysis was carried out by RDP II and MEGA6. Finally, the production of protease, lipase, urease, cellulase and xylanase was detected using the appropriate substrates under acidic conditions. **Results:** mine tailings have high concentrations of sulfate, Zn, Pb, As, Cd, Ni and Ag. 20 bacterial strains were isolated and belonging to genus of *Bacillus*, *Pantoea*, *Enterobacter, Oceanobacillus, Burkholderia* and *Streptomyces*. In regard to the enzymes, 43% of the strains produced lipase and protease, 26% cellulase and 13% urease and xylanases. **Conclusion**. Bacteria isolated from mining tailing are able to grow in acidic pH and tolerate heavy metals, besides produce various exoenzymes, which have potential applications in the biotechnology and industrial fields.

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# Interaction proteomics of Op14-3-3mu protein of *Opuntia ficus indica*

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Protein-protein interactions are essential biological process involved in several cellular functions. In eukaryotes exist a conserved family of proteins, named 14-3-3, which have the ability to bind to phosphorylated proteins. Due this property, 14-3-3s are involved in several relevant processes for the cells, such as activation/deactivation of enzymes, the translocation into/out of organelles, transcription and signal transduction. In plants, 14-3-3s are related to plant stress responses through interactions with key proteins of metabolism, light and hormone signalling, cell-cycle control, and protein trafficking [1]. Specifically in plants, 14-3-3s appear to regulate phosphate uptake [2]; moreover, in processes involved by gibberellin and abscisic acid, they function as phospho-sensors [3]. 14-3-3s have the ability to form heterodimers among different isoforms and to bind simultaneously to either two targets or two regions of the same target. In *Arabidopsis*, 13 unique paralogs exist and can be subdivided into two distinct subgroups, epsilon and non-epsilon. Recently, *Opuntia* shotgun proteomics have revealed the presence of 14-3-3s proteins [4]. *Opuntia* spp. are crassulacean acid metabolism (CAM) plants, associated with the semi-arid areas and *O. ficus-indica* is the species with the highest economic importance [5].

In the present work, an orthologous of the At14-3-3mu, named Op14-3-3mu, was cloned and recombinant protein was expressed in a heterologous system. In order to know more about Op14-3-3mu the biochemical characteristics such as thermal stability, dimerization ability, and molecular weight were investigated. Using proteomic tools was identified other 14-3-3 that interacts with Op14-3-3mu and other proteins clients of Op14-3-3mu. This is the first report that is carried out the characterization of a 14-3-3 protein in nopal and is relevant since its high agrifood value and its ability to grow in abiotic stress.

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# Study of expression of *pvdS* and *csbC* genes in *Azotobacter vinelandii*

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Siderophores are low molecular weight compounds capable of solubilizing and chelate iron and they are classified in order to functional group that interacts with iron, in three types: carboxylates, hidroxamates and catecholates, also there are mixed siderophores which have different type of functional groups. In *Pseudomonas aeruginosa,* transcription of genes involved in pyoverdine (mixed siderophore) production is activated by a sigma factor encoded by the *pvdS* gene, and regulation of *pvdS* is related to Gac-Rsm regulation pathway, which includes the two-component GacS/GacA system and a post-transcriptional control system comprised of Rsm small regulatory RNAs and the RsmA repressor protein. *Azotobacter vinelandii* belongs to *Pseudomonadaceae* family and produces pyoverdine at concentrations ≤ 3 μM of iron. Also, this microorganism has homologous genes to those of *Pseudomonas aeruginosa* that are involved in pyoverdine production, including *pvdS* and *pvdI,* which encode for the transcriptional regulator and a non-ribosomal peptide synthetase, respectively. In addition to pyoverdine, *A. vinelandii* produces four catecholate siderophores (azotochelin, aminochelin, protochelin and DHBA or dihydroxybenzoic acid) that are synthetized at concentrations ≤ 10 μM of iron. About the genes involved in catechols synthesis, is known that the gene encoding for isochorismate synthase enzyme, *csbC,* is the key in catechol production.

In *A. vinelandii*, GacS/GacA-Rsm regulatory system is involved in the regulation of pyoverdine production, GacA mutants do not produce pyoverdine and increase catechol production compared to wild type. The main objective of this work is to study the expression of *pvdS* and *csbC* genes by using transcriptional and translational fusions with the reporter gene *gusA* to study the effect of the mutation in *rsmA* on the expression of pyoverdine and catechol biosynthetic genes. Therefore, the integrative vectors pUMATcgusAT and pUMATcgusAPT were used to clone the *csbC* regulatory region (217 bp) as well as the regulatory regions of *pvdS* and *pvdI* (435 bp). The regulatory regions were amplified with primers containing artificial restriction sites *Sac*I to subsequently clone it in the aforementioned vectors. Once obtained the transcriptional fusions *pvdS-gusA, pvdI-gusA* and *csbC-gusA,* and translational fusions *pvdI-gusA* and *csbC-gusA* were used to transform *A. vinelandii* wild type strain and its derivate *gac* and *rsm* mutants and subsequently to measure enzymatic activity of β-glucoronidase to determinate the regulatory relation between the Gac-Rsm pathways with the siderophore synthesis in *A. vinelandii*.

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# Partial Characterization of Antimicrobial Substances Produced by *Lactobacillus paracasei* KSI

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**Abstract.** The genus *Lactobacillus* are Gram positive bacilli belonging to the group of lactic acid bacteria (LAB). They can be found colonizing the digestive tract and vagina of many mammals including humans. Their use as probiotics is given by their influence on the intestinal microbiota and their antagonism effect over pathogenic bacteria is due, especially, to produce antimicrobial substances called bacteriocins, which can help to combat and prevent infection in their hosts. The aim of this work was the partial characterization of antimicrobial substances produced by the strain of *L. paracasei* KSI and evaluation of their antimicrobial activity. Methodology: The isolated strain was identified microbiologically and genetically by sequencing the *r16S* gene and housekeeping genes (*hsp, recA, rpoB*). Antagonism tests were performed by the agar well diffusion method, using cell free extract from the isolated strain vs several reference strains. The extract was submitted to several conditions of temperature (121 °C/15 min; 100 °C/30 min; 37 °C, 25 °C, 4 °C, -10°C and -80 °C/7 days), pH (4 to 8.8), enzymatic digestion (Proteinase K, Trypsin and Lysozyme), ultrafiltration (3, 10, 30 and 50 KDa membranes) and extraction with different organic solvents (chloroform, hexane, ethyl ether, ethyl acetate, n-butanol and isopropanol). Results: *L. paracasei* strain was identified and called KSI. The cell-free extract showed inhibitory effect against the tested strains *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, but not against *S. aureus* ATCC 25923. The extract showed thermal stability maintaining its inhibitory effect. It was demonstrated that every filtered had the same antagonistic effect; however, when the cell free extract was concentrated by evaporation under reduced pressure, an increase of inhibitory effect occurred even against *S. aureus*. Similarly, the activity of the extract was observed to pH=4, but not in higher values than pH=5; this effect was preserved after enzymatic treatment. In other hand, from the extraction with ethyl ether, ethyl acetate and n-butanol, active fractions were recovered. Conclusions: *L. paracasei* KSI strain generates more than one type of bioactive molecule, of different sizes, stable to thermal and digestive treatments, but unstable at pH upper 5, with a potential biotechnological application.

**Keywords:** *Lactobacillus paracasei, Probiotic, Bacteriocins, Antagonism, Bioactive Molecule.*

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# Characterization of mutant $PilR_{D53N}$ in the two component system PilS-PilR of *Geobacter sulfurreducens* and its relationship with the electron transfer.

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Geobacteraceae is the predominant group of bacteria in subsurface environments, where dissimilatory metal reduction is an important process. *Geobacter sulfurreducens* is a Deltaproteobacteria, a member to the Geobacteraceae family. This group of bacteria lives in the subsurface where they have the ability to transfer electrons to extracellular acceptors as to Fe(III), Mn(IV) oxides, U(VI), humic substances and electrodes (Lovley et al. 1996). Due to this capability, Geobacter has an important role in biogeochemical cycles of metals like Fe y Mn, in nowadays it has found its application in the bioremediation of contaminated environments with organic matter and heavy metals and the generation of bioelectricity in microbial fuel cells (MFC) (Lovley 2006, 2011).

In *G. sulfurreducens*, pili are essential for long-range extracellular electron transfer to insoluble Fe(III) oxides and are also required for transfer the electrons to the anode in MFC. The type IV pili of these bacteria are formed of monomers of pilin or PilA, encoded by the *pilA* gene (Reguera et al, 2005, 2006). The *pilA* transcription is mainly driven by the RNA polymerase with σ54 factor (RpoN) and the transcriptional regulator PilR. Is well know that the σ54 factor requires the activators EBP type (Enhancer Binding Protein) to recognize and activate its target genes. PilR is a regulator EBP type and it is also a member of Two Component System (TCS) where PilS is a Histidine kinase (HK) and (PilR) is the Response Regulator (RR).

It has been studied the TCS PilS-PilR of *G. sulfurreducens* in heterologous expression system in *Escherichia coli.* We determinate the phosphorylation sites in both proteins, His-334 residue conserve in PilS and Asp-53 residue conserved in PilR; with Electrophoretic Mobility Shift Assay it was established that PilR active directly the expression of *pilA,* to bind to the promoter region of *pilA.* It is also known that the D53N substitution in PilR, which favors the unphosphorylated status, increases the expression of PilA more than wild type protein. This was relevant, because there are few examples where the unpohosphorylated RR is the form active. It is known that increased production of pili, improve the electron transfer to metals and electrodes. Therefore, we are studing the mutant $PilR_{D53N}$ in order to compare the phenotype obtained in the heterologous host, where this mutant increased expression of PilA, and therefore we could observe more electron transfer and production of bioelectricity in MFC.

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# Micropropagation of *Mammillaria rhodantha* in temporary immersion system

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Cactaceae is a succulent plant family of great diversity in Mexico. However, many of them, including *M. rhodantha*, are extinction-endangered caused mainly to the loss of their habitats and the illegal and excessive collect of wild specimens for ornamental purposes. For that reason, it is important to develop efficient protocols for its propagation using *in vitro* plant tissue culture technics. Temporary Immersion Systems (TIS) consist of devices that permit the intermittent contact of plant tissue with the liquid culture medium, allowing the advantages of liquid culture and eliminating the drawbacks of continuous contact in regular plant bioreactors. TIS also present technical and economic benefits for scaling up and automation. The aim of this project was to evaluate the feasibility of TIS for the *M. rhodantha* micropropagation and establishing the optimal conditions for this purpose. Plants, *in vitro*-germinated, were tested to different combinations of plant growth regulators, both in semisolid and liquid medium culture. It was found that using the TIS, plants grew up to twice bigger in the presence of 10 mg/L bencyladenine, and the absence of auxins, compared to semisolid culture. The optimization of frequencies and duration of tissue immersion in the liquid medium showed better results with 10 minutes of immersion every 24 hours, considering the growth of plants and the absence of hyperhydricity.

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# PRODUCTION AND PURIFICATION OF THE LEUCIN-AMINOPEPTIDASE yspII PRODUCED IN THE YEAST *Pichia pastoris*

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The LAPs proteins belong to families of proteases M1 and M7 are a group of zinc- and manganese-dependent metallopeptidases. They have various functions such as inactivation of polypeptides, regulation of meiosis and are present in all the stages of growth of some parasites, by which they were proposed as potential targets for vaccines. In our laboratory in 2007, it was isolated from yeast Schizosaccharomyces pombe a new cytosolic exopeptidase, called "leucine aminopeptidase yspII" (**LAPyspII**), which is dependent on $Mn^{2+}$, belongs to family , it is an hexameric protein with a molecular mass of 340 kDa and six subunits of 54 kDa. *In silico* modeling shows a saddle-shape α/β motif. The active site is located at the C-terminus also has a regulatory site with $Zn^{2+}$ or $Mn^{2+}$ ions, it was found that the Lys-292 is fundamental in the catalysis.
For progress in the molecular characterization, producing strain LAPyspII was obtained from the yeast Pichia pastoris, as it is a very efficient system of overexpression of heterologous proteins. The aim of this work was the production of recombinant enzyme LAPyspII from Pichia pastoris, purification by Immobilized-metal affinity chromatography (IMAC), and characterization of kinetics parameters.
It started with a strain of P. pastoris overproducing the recombinant enzyme LAPyspII (X-33LAPII). In the AOX1 gene of chromosome, was inserted the ORF of ape2 gene, with a selection zeocin marker, an alpha factor that allows the protein of interest is expelled extracellularly, one epitope Myc for identification by Western blot and one motif for histidines (6X) for purification. The Ape2 gene expression and production of the LAPyspII is obtained by the strain growth with methanol as the sole carbon source. It is observed that the cells grew slowly with methanol, and increased production was obtained on the sixth day in a cell-free growth medium. LAPyspII recombinant enzyme was concentrated by precipitating with ammonium sulfate to 80% saturation and loaded onto affinity column Ni-NTA (IMAC). By gel electrophoresis SDS-PAGE and Western blot was observed that the protein eluted with high efficiency and with a good degree of purification. The LAPyspII recombinant enzyme had a good enzymatic activity and kinetic parameters (Km and Vmax) were very similar to the native LAPyspII.

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# Interaction between Bcl-2 and Bax proteins.

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All multicellular organisms must have an exquisite regulation of their cell mass turnover during all their life span in order to avoid a variety of severe health disorders. Apoptosis is the most common programmed cell-death process within mammals. Members of the Bcl-2 protein family accurately regulate the apoptotic process through interactions with several proteins including members of the same family which dictate the integrity of the outer mitochondrial membrane (OMM), and in consequence the cell's fate to live or die. The Bcl-2 pro-survival proteins function mainly by constraining activation of pro-apoptotic members of the family. In the other hand, pro-apoptotic members induce apoptosis by triggering destabilization of OMM and consequently releasing the apoptogenic-factors, like cytochrome C. Bcl-2 and Bax represent the archetypical members of anti- and pro-apoptotic groups, respectively . The way these two proteins interact with each other to regulate apoptosis remains as an unsettled central issue. We have built two models of the complex formed by the full-length Bcl-2 protein and the BH3 domain of Bax by two different techniques of molecular modelling. In this work we describe at atomistic level these two models. These results might have important implications in understanding interactions between members of the Bcl-2 family, and might be useful in rational drug design.

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# Central Nervous Systems Effects of Nanoparticles Administered Orally

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Abstract

Currently, attention has focused on nanoparticles as possible vehicles of drugs with low oral bioavailability. Among the nanoparticles of interest are nanoparticles of silicon dioxide ($SiO_2$) as they have advantages as a highly porous structure, high surface area, easy resizing and pore volume, apparent biodegradability and biocompatibility, excellent ability to enhance the solubility of poorly soluble drugs, stable drug loading in its pores. So $SiO_2$ nanoparticles have become an ideal drug delivery vehicle. However, its effects in the central nervous system (CNS) are still unknown. To be used as a vehicle for drugs whose destination is the CNS, it is necessary to evaluate their possible effects in healthy and oxidative conditions of CNS.

This study evaluated the effect of $SiO_2$ nanoparticles in two brain regions (striatum and substantia nigra) in dopamine levels, lipid peroxidation, and mitochondrial function under physiological and toxic conditions in the model of MPTP-induced Parkinsonism.

$SiO_2$ nanoparticles of 200 nm diameter in three doses (25, 50 and 100 mg/kg body weight) were administered for five consecutive days in healthy mice and mice exposed to MPTP. HPLC determined dopamine levels; lipid peroxidation was evaluated by measuring malondialdehyde (MDA) levels, and mitochondrial function by MTT reduction.

Our data show that none of the three doses tested of $SiO_2$ nanoparticles modified the levels striatal dopamine, also did not alter the mitochondrial function nor induced lipid peroxidation in any of the two brain regions evaluated.

Under oxidative conditions, the administration of $SiO_2$ nanoparticles did not further modify de reduction of mitochondrial function and lipid peroxidation induced by MPTP administration in the two evaluated brain regions.

Our data demonstrate that $SiO_2$ nanoparticles may be a possible vehicle for CNS drugs since they lack adverse effects in the CNS.

# Determination of growth kinetic parameters of the plant pathogen *Ralstonia solanacearum*

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In Mexico, the production of tomato ascends to around 15 thousand millions of Mexican pesos per year (SIAP/SAGARPA, 2013) and is one of the most important agricultural products in the world. Several pathogen agents like fungi, bacteria and viruses, affect its cultivation causing significant economic losses.

*R. solanacearum* is known to cause bacterial wilt disease in tomato plantings. In 2012, Hernández-Romano, J., *et al*, reported the isolation of this bacterium from tomato plants of several greenhouses in Morelos, Mexico. Thus, the necessity to develop a sustainable agriculture has leaded the search of new control agents. According to this, our research group has isolated several lytic bacteriophages in order to combat wilt disease. The evaluation of the protective effect of bacteriophages against *R. solanacearum* in experimental greenhouses, as well as the possible industrial production of them, require the implementation of bioprocesses that will generate large volumes of phage solutions. However, since phage therapy is an approach that has emerged in recent years, large-scale production processes are still in development. Thus, the initial effort in process development involves the measurement of kinetic and stoichiometric parameters of *R. solanacerum* grown in minimal medium with glucose as sole carbon source. Main results of this study will be showed and discussed during the meeting. In our knowledge, this is the first time that these parameters are measured for *R. solanacearum* and represents an important step to improve the production of this agricultural product.

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# Heavy metal-resistance profile of *diazotrophic bacteria* isolated from root of *Acacia farnesiana* (L) Willd that grow in acidic mine tailings

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**Keywords:** pioneer plants, *Acacia farnesiana*, CMI, Phytoremediation

**Introduction:** Mining generates a lot of toxic tailings and these are the most important source of emissions of heavy metals which represents a serious problem of contamination to the environment and human health. In Taxco de Alarcon, Gro., the mining tailings El Fraile is characterized by extreme acidity and high concentration of Fe, Pb, Zn and As. However, several genres of plants are able to grow in this area including *Acacia farnesiana* (L) Willd capable of stabilize As, Pb, Fe, Cd, Zn (Olea-Garcia and Zúñiga-Ocampo, 2009). Various studies suggest that processes metal tolerance by plants are further enhanced by bacteria that inhabit its root and rhizosphere due to production of phytohormones, secondary metabolites and enzymes that promote plant growth. Moreover, they have mechanisms for biotransformation of metals decreasing toxicity and mobility of these elements, so that they could help in the restoration and revegetation of mining areas. Therefore, the **aim** of this study was to isolate diazotrophic growth promoting bacteria from root of *Acacia farnesiana* to evaluate their biofertilization mechanisms and heavy metal resistance profile. **Methods:** diazotrophic bacteria were isolated from the root of *Acacia farnesiana* in nitrogen-free medium. Different plant growth-promoting activities such as auxins, gibberellins, and solubilize phosphorous were determined in all the isolated bacteria. The *nifH* and *acdS* gene encoding nitrogenase and 1- aminocyclopropane-1-carboxylate (ACC) deaminase were detected respectively. Finally, the minimum inhibitory concentration (MIC) was determined to 17 metal salts: $Pb(NO_3)_2$, $PbCO_3$, $FeSO_4$, $FeCl_3$, $NiCl_2$, $CoCl_2$, $CuCl_2$, $CdCl_2$ $HgCl_2$, $Na_2CrO_4$, $K_2Cr_2O_7$, $CrCl_3$, $AgNO_3$, $NaAsO_2$, $Na_3AsO_4$, $ZnCl_2$ y $H_3BO_3$. **Results:** 20 strains diazotrophi**c** were isolated, which the 40% produced auxins, 85% gibberellins and none were capable of solubilizing phosphates. The *nifH* gene was detected in all strains and *acdS* gene only in the 75%. the following profile of MIC was: $Hg^{2+}$ (200 ppm), $Cr^{6+}$ (750 ppm), $Pb^{2+}$(800 ppm), $Ag^{2+}$(1200 ppm), $Cd^{2+}$(1600 ppm), $B^{3+}$(2000 ppm), $As^{3+}$(3000 ppm), $Co^{2+}$(4000 ppm), $Ni^{2+}$(4000 ppm), $Cu^{2+}$(4000 ppm), $Zn^{2+}$(4000 ppm), $Fe^{2+}$(4000 ppm), $Cr^{3+}$(>4000 ppm), $Fe^{3+}$(>4000 ppm), $As^{5+}$(>6000 ppm). More tolerant strains were ERH6, ERH11, ERH12, ERH25 and ERH34, which showed morphological changes depending on the metal in which they grew. **Conclusion:** The isolated bacteria are able to fix nitrogen, producing phytohormones and resistance to several heavy metals, so they might be favoring the growth of plants and stabilize heavy metals on mine tailings.

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# Hydrogen production by strain G088 psicrófilic using a dark fermentation process from agro-industrial waste

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The depletion and the increased cost fossil fuels has come to explore new alternative energy. Biohydrogen gas high energy efficiency is considered as the source of clean, renewable energy future. Research shows that biohydrogen production from agro-industrial waste using mesophilic and thermophilic microorganisms are the disadvantage of a high energy cost for operating conditions. In this work an isolated strain of Antarctica G088 (related to *Polaromonas* genus) than in previous studies that have demonstrated the capacity to produce biohydrogen from simple carbohydrates. The aim of this work was to optimize conditions for biohydrogen production using cheese whey powder as the sole carbon source. An central composite design $2^3$ were applied to find influence of three variables of process on which includes pH, temperature and concentration of cheese whey powder for biohydrogen production. The conditions optimum of biohydrogen production showed a yield of 4.2 mol $H_2$/mol lactose. The results showed that psychrophilic bacteria G088 has great potential for the production of biohydrogen.

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# Effect of textile dyes on growth of *Pleurotus ostreatus* and production of dye peroxidase

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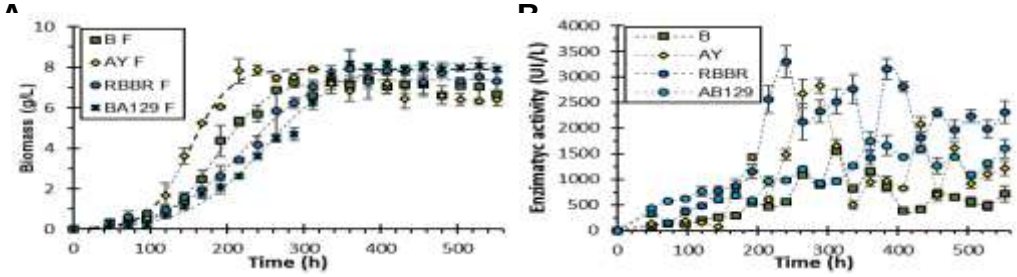
The white rot fungi basidiomycetes offer a biotechnological alternative for xenobiotics degradation and other compounds. *Pleurotus ostreatus* has been a model of research focused on the production of peroxidases involved in such processes[1].

In this work, the effect of three dyes with different chemical structures on growth of *P. ostreatus* and production of dye peroxidases is analyzed.

Submerged fermentation in basal medium[2] and supplied with 500 ppm of dye azo yellow (AY), remazol brilliant blue R (RBBR) and acid blue 129 (AB129) was realized, they were incubated to 25 °C and 120 rpm. The maximum of biomass (Fig 1A) was obtained in the fermentation with AB129 (7.37 g/L), while the minimum of biomass was presented in basal fermentation. On the other hand, the fermentation with AY had the highest growth rate (0.0389$h^{-1}$), while the lower growth rate was obtained in the fermentation with AB129 (0.0199 $h^{-1}$).

The enzymatic activity was measured by ABTS oxidation[3] in tartrate buffer pH 3.0 and incubated to 45 °C. The highest activity (Fig 1B) was obtained in the fermentation with RBBR (3299 UI/L) to 240 h, while the minimum activity occurs in the fermentation with AB129 (1744 UI/L) to 360 h.




The presence of dyes in submerged fermentation modified the growth kinetic parameters of *P. ostreatus*, such the DyP production, showing a clear effect on induction of enzymatic activity. This research was supported by grant SIP20161426 from Instituto Politécnico Nacional.

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# Chlorophyll A extraction from *Arthrospira platensis* and evaluation of its activity as photosensitizing agent in *Staphylococcus aureus.*

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Chlorophylls represent an important kind of organic photopigments, with potential applications in pharmaceutical, cosmetic industry and several fields. Particularly, they are considered good candidates as antimicrobial photosensitizing agents, for inhibition of some bacteria like *Staphylococcus aureus;* due its structural malleability, photophysical and photobiological properties, and its high level of functionalization. There are several methods for extraction and isolation of chlorophylls. Although these processes usually becomes expensive or non-profitable, and even though not fully extracted or pure. Thus, the objective of this work is to propose a new methodology for optimal extraction, purification of chlorophyll A and the evaluation of its activity as photosensitizing agent in *Staphylococcus aureus*. *Arthrospira platensis* was used for chlorophyll A extraction, first step of purification was done by column chromatography with a mobile phase composed of ethyl acetate, dichloromethane and toluene; which turned out to be efficient and selective for chlorophyll A. Subsequently, a characterization by Ultraviolet-Visible Spectroscopy (UV-VIS), Infrared Spectroscopy (IR) and Liquid Chromatography-Mass Spectroscopy (LC-MS) was performed to corroborate chlorophyll A purity. Results showed an efficient and selective extraction for isolation and purification of Chlorophyll A. For photosensitizing assays four experimental groups were used in antimicrobial activity: Control (AFS cells and without light), illuminated (cells without AFS and radiated with LED light), obscure (incubated cells with AFS and with no LED radiation) and TFD (incubated cells with AFS and radiated with LED light). Antimicrobial activity on *S. aureus* was detected. It can be concluded that our method for chlorophyll extraction is simple and less expensive than conventional methods. Besides chlorophyll extracts obtained by this method are useful as a photosensitizing agent against *S. aureus*.

# Functional analysis of a red-far red light photoreceptor in the filamentous fungus *Trichoderma atroviride*.

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*Trichoderma* spp are cosmopolitan filamentous fungi commonly found in soil, which are best known for their ability to antagonize phytopathogenic fungi. Currently, to protect crops it is dispersed in the field as conidia formulations. For that reason, the mycoparasitic fungus *T. atroviride* has been using as morphogenetic model to study conidiation stimulated by light, injury, stress, and nutrients deprivation.

Light apart from being one of the fundamental type of life energy on the earth, it also has information for living forms, playing a critical role in the behavior of plants, bacteria and fungi. It has been described its intervention in growth and pathogenicity as well as the balance between sexual and asexual reproduction, direction of growth and formation of pigment, all of this factors are relevant for the survival of the species.

The photoreceptors are proteins bound to chromophores that have the capacity to respond to different wavelengths of light. The fungi have several photoreceptors, which would point to a complex light sensor mechanism in these organisms. As described in fungi and other organisms, like plants, the role of red and far-red light has been described on asexual sporulation, germination and growth. *T. atroviride* has a homologous gene encoding a protein that contains the typical phytochrome domains, named *phy*1.

In this work, mutants deleted in *phy*1 were obtained by the double homologous recombination method and the PEG (Polyethylene glycol) method. The mutant strains were corroborated by PCR with specific primers to detect the gene replacement event. Analysis of conidia germination using different wavelengths indicates that solely blue light negatively regulates this process. The mutants lacking in phy1 showed a faster germination under constant blue light than the wild type strain, suggesting that the light effect was lost in the mutant strains. These results suggest that Phy1 has overlapped its signaling with other wavelengths or photoreceptors.

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# Antagonistic potential of bacteria isolated from agricultural soils in the state of Nayarit against pathogenic fungi of agronomic importance

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The antagonistic potential of bacterial isolates obtained from agricultural soils in the state of Nayarit was evaluated by microbiological, biochemical and molecular analyses. Fifteen bacterial isolates previously obtained on basis to preliminary screening that suggested their potential antagonistic against phytopathogenic fungi, were used in this study. The biochemical assays evaluating antifungal activity traits such as hydrolytic enzymes (chitinase, protease, cellulase, pectinase, amylase) and siderophores production showed that ZJ1, ZJ2, ZJ4, ZJ7, ZJ157 and ZJ168 strains presented the majority of the evaluated traits. *In-vitro* assays for antagonism against *F. oxysporum*, *F. stilboides, Verticillum* and *P. expansum* revealed significant inhibitory effects on mycelial radial growth by all the six isolates. These inhibitory effects appear to be due to the presence of volatiles compounds and/or extracellular filtrate compounds. The molecular analyses showed that only the ZJ7 and ZJ157 strains content genetics determinant characteristic of antagonistic bacteria. The molecular identification indicates that the isolates belong to Bacillus spp. and Pseudomonas spp. These results exhibit the antifungal activity of bacterial isolates and indicate the possibility of using these as antifungal against these fungal species.

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# CRECIMIENTO DE *Pleurotus ostreatus* ATCC 32783 Y ACTIVIDAD DE LACASA INTRACELULAR DESARROLLADO A DIFERENTE pH INICIAL DE DESARROLLO EN FERMENTACIÓN LÍQUIDA

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*Pleurotus ostreatus* es un hongo basidiomiceto que produce múltiples isoenzimas dependiendo de las condiciones de desarrollo (1). La producción de lacasas está influenciada por factores ambientales como el pH, temperatura, tipo de cultivo y composición del medio (2). *P. ostreatus* en medio líquido estimula la producción de lacasas extracelulares siendo mayor con respecto a la obtenida por medio sólido (3). Por lo que en este trabajo se evaluó el efecto del pH sobre el crecimiento y actividad de lacasas intracelulares de *P. ostreatus* desarrollado en fermentación líquida (FL). Se realizaron FL en matraces Erlenmeyer de 125 ml con 50 ml de medio de cultivo. El pH inicial se ajustó a 3.5, 4.5, 6.5 y 8.5, se inocularon tres fragmentos de micelio de *P. ostreatus*; se obtuvo la biomasa por filtración, el muestreo se realizó cada 24 h hasta la fase estacionaria. Del sobrenadante para cada fermentación se evaluó la actividad de lacasas intracelulares por espectrofotometría utilizando 2,6 Dimetoxifenol (DMP) como sustrato. El crecimiento de *P. ostreatus* en FL, obtuvo una X*max* de 5.2, 5.5, 9.6 y 8.3 g/L, y los valores de μ fueron de 0.006, 0.014, 0.018 y 0.02 h-1 en los cultivos a pH inicial de crecimiento de 3.5, 4.5, 6.5 and 8.5, respectivamente. Por otro lado, las actividades intracelulares de lacasa de las FL de pH de 3.5, 4.5, 6.5 y 8.5, tuvieron valores máximos de aproximadamente 817, 4800, 1741 y 532 U/g de biomasa seca respectivamente.

A manera de conclusión podemos sugerir que El pH es un factor importante en la velocidad específica de crecimiento (μ), producción de biomasa (X) y en la actividad enzimática de lacasas intracelulares. Este es el primer reporte sobre el efecto del pH inicial de crecimiento de *P. ostreatus* sobre el efecto en la actividad intracelular de lacasas. La fermentación a pH inicial de 4.5 mostró los valores más altos en la actividad de lacasa intracelular.

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# Streptomycin detection in wastewater by dot blot

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There is now a growing interest in emerging pollutants (EC), which are compounds of different origin and chemical nature, whose presence in the environment, have gone largely unnoticed, causing environmental problems and health risk. Among emerging contaminants are antibiotics, which are released into the aquatic environment, putting on risk the ecosystem and the public health. These contaminants are at low concentrations, they are not regulated, but may be candidates for future regulation. Among these EC are those from pharmaceutical origin as the case of antibiotics because of the problems presented on their removal, there is a potential danger in generating bacterial strains of different microorganisms with resistance to antibiotics. A widely used antibiotic is streptomycin (STR, aminoglycoside antibiotics), which has been reported to cause ototoxicity and nephrotoxicity problems among others, in humans.

The EC could in the future be regulated for which it is necessary to know its presence through monitoring, however, the methodologies used for the detection of antibiotics in environmental matrices require handling complex, costly equipment, time-consuming in their management, plus its operation is not simple. An option to these methodologies are those based on immunochemical interactions, which have shown good results, easy development and high detection sensitivity, are those based on immunological interactions between antibody specifically directed against these drugs. Such methodologies allow processing high numbers of samples and the use of small volumes of the same, due to the specificity between the antibody- antigen.

Because of this, this work focused on developing a dot blot with specific polyclonal antibodies against STR which were previously obtained. The lowest limit detection presented by these anti-STR was 25 μg / ml (0.0025%). However, these antibodies were not specific only against the STR, but presented a cross reaction against other aminoglycoside antibiotics (kanamycin, neomycin and paromomycin), but not aminoglycoside antibiotic. This, far from presenting a problem, brings the advantage of a greater number of detectable molecules in wastewater. It is noteworthy that most aminoglycoside antibiotics are used in combination, therefore, the presence of only one these in wastewater isn´t viable. As antibodies able to recognize other antibiotics, the uses theses in tests are extended to a large range of more molecules of interest.

With antibodies obtained in the laboratory, it was possible to detect STR in wastewater adulterated in the laboratory. With this, it was shown that uncontrolled physical and chemical conditions in which the water is, it does not affect the reactivity of antibodies against streptomycin.

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# Biopreservation of food pork ready to eat meat by lactic acid bacteria

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Consumers are becoming more aware of additives around the world. They are expecting, ready-to-eat meals that can offer a healthy option, which means less additives and the use of functional ingredients. Prebiotics, in particular, chicory inulin has proven to meet these requirements. According, to Mexican Regulations, when a prebiotic is present in a 2.5% ratio, the food product is considered a good source of fiber.

On the other hand, It has been reported, in seafood, that lactic acid bacteria when added to packed products can compete with bacteria responsible of spoilage, and also during growth it can produce: lactic acid, hydrogen peroxide, bacteriocins, and other type compounds. Therefore, biopreservation through the use lactic acid bacteria for decreasing preservatives and for increasing shelf life could be a novel solution in ready to eat pork meat products. On the other hand, some lactic acid bacteria have the characteristic of being probiotics. And according to Mexican Regulations, when present in a ratio of $1 \times 10^{8}$ UFC/mL, a food product can be considered a probiotic one.

On this study, a ready-to-eat marinated pork product designed with chicory inulin in a 3% was used to incorporate three different lactic acid bacteria in a 5% ratio: *Lb. delbrueckii* subsp. *bulgaricus* NCFB 2772, *Lb. rhamnosus* GG and the combination of both bacteria. It is important to mention that *Lb. rhamnosus* GG has proven have probiotic character.

After preparation, the product was stored at 4°C and microbiological, physicochemical and sensory parameters were analyzed every week over a month storage period. Lactic acid bacteria, aerobic mesophilic bacteria and total plate count were analyzed for the microbiological study. Additionally, physicochemical parameters analyzed were: texture profile analysis (TPA), color, and pH. Lastly, a sensory analysis using triangle test was used in order to show when differences were perceived by a trained panel based on odor and color, results were analyzed by through IFPrograms™.

Results show that the marinated pork product can be considered a good source prebiotic source. Additionally, biopreservation can be demonstrated because there is an inhibition present. During the period of storage the amount of lactic acid bacteria was not enough to consider the product as a probiotic one. However, the product can be considered safe and adequate for scaling up.

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# Identification of structural genes of enterocin 29α and enterocin 29β in the operon of the enterocin A produced by the strain *Enterococcus faecium* MXVK29

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Key words: Bacteriocins, *Enterococcus*, Fermented sausages, Operon.

The genus *Enterococcus* comprises a group of microorganisms used as starter cultures or probiotics. They inhibit the damaging/pathogenic biota present in food and are an important part of gastrointestinal tract in human, because prevent tract colonization by harmful bacteria. They have also the ability to produce some antimicrobial products such as lactic acid or bacteriocins. *Enterococcus faecium* MXVK29 isolated from fermented sausages produces a bacteriocin of 3.5 kDa, which belongs to class II.1, according to its amino terminus sequence, and is active against strains of *Listeria sp.* and *Staphylococcus sp.* The objective of this work was to identify the genes that form the operon of the bacteriocin of class II.1 produced by *Enterococcus faecium* MXVK29. The genomic DNA of all the strains was extracted from 18-h cultures using a commercial kit. PCR was performed using different sets of structural genes of enterocins. Inverse PCR and Primer Walking were realized to identify adjacent genes. The fragments were sequenced and compared in the database of NCBI. The bacteriocin produced by *Enterococcus faecium* MXVK29 had a similarity of 100% with enterocin A. Furthermore the adjacent genes identified for this bacteriocin are immunity (*entI*), induction (*entf*), the accessory genes (*entK, entR and entT*), as well as three ORFs that have a high identity with class IIb bacteriocins. *Orf 2* (29α) shows a consensus motif GxxxG similar to those shown by bacteriocins as PlnNC8α, EntCα, and Ent1071α, while *Orf 3* (29β) shows a consensus motif similar to that present PlnNC8β or PlnCβ (AxxxA). These kind of bacteriocins are only express in co-cultures, so there is the possibility that expression of this bacteriocin of two peptide could be induced by a similar mechanism. More studies are needed to know the possible induction, function and hypothetical origin of these genes.

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# Physiological effects caused by reduction on glucose uptake capacity in *Escherichia coli* mutant strains devoid of glucose transport related genes.

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**Background:** *E. coli* can grows in glucose-supplied media, by internalizing sugar through the phosphoenolpyruvate:sugar phosphotransferase system (PTS). This system couples transport and phosphorylation of sugars and is composed by enzime I, phosphohistidine carrier protein, and enzymes IIA and IIB. Additionally, the PTS components IIC, and some cases IID constitute integral membrane protein permeases. Transport systems related with galactose transport (non-PTS transporters) as well as the galactose:$H^+$ symporter GalP or the high-affinity ABC transporter Mgl system these both are induced and work as glucose transporters when *E. coli* grows under either glucose limitation or PTS inactivation (Fuentes, 2013; Hernández-Montalvo, 2003). *E. coli* displays a high growth rate and a high consumption rate under aerobic and glucose non-limiting conditions. Under these conditions, acetic acid is produced as a consequence of metabolic imbalance between acetil-coenzyme A (AcCoA) synthesis and consumption. Acetic acid is a problem since it represents a carbon loss, reduces pH in culture medium, and can be deleterious for cells (Fuentes, 2013; De Anda, 2006). In our group, Fuentes studied sugar transport contribution in variations of genes encoding PTS and non-PTS transporters, by generation of 15 isogenic *E. coli* W3110 derivatives in single or multiple deletions. These strains displayed a wide variety of specific growth rates (μ), spanning from 0.65 to 0.18 $h^{-1}$, and specific glucose consumption rates (qs) on a range from 1.33 to 0.32 g/g DCW h. Furthermore, Soto R, *et al.* 2011 used VH33 strain, an isogenic derivative strain of *E. coli* W3110 devoid of genes encoding PTS proteins. For VH33, glucose transport depends on GalP and has 42% lower qs than W3110 strain.

**Results:** We chose seven different mutant strains from the collection used by Fuentes and Soto to test physiological effects related to the reduction of glucose uptake capacity in *E. coli* mutant strains deficient of glucose transport related genes. We tested these strains in M9 minimal medium with 20 g/L of glucose, into 1L bioreactors, under controlled conditions of aeration, pH, dissolved oxygen and temperature. Under these culture conditions we observed that strain collection displayed different μ, spanning from 0.67 to 0.23 $h^{-1}$, and different qs spanning from 1.78 to 0.23 g/g DCW h. These results are very similar with that reported by Fuentes. On the other hand, specific acetic acid production rate was almost 10-fold in W3110 strain, related to the other strains.

Aditionally, we evaluated transport and glucose metabolism expression, from a set of genes. We measured *ptsG, manX, ptsI*, which encode two PTS permeases and a PTS component respectively. We also measured the expression of non-PTS transporters GalP and MglB and glucokinase encoded by *glk*. We have found that genes related to transport of alternative carbon sources *manX* and *mglB* had 8 and 40-fold overexpression level in WG strain. This response was consistent with results published by Nahku R, *et al*. 2010. The genes *ptsI, galP* and *glk* had the same expression level in both strains. In WG strain, we did not observe *ptsG* expression due to the absence of this gene.

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# Growth assessment of *Rhizobium sp.* in minimal medium with glucose or succinate as carbon sources

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## Abstract

*Rhizobium sp.* is a rhizospheric bacteria able to realize biological nitrogen fixing when establishes a symbiotic relationship with the common bean plant *Phaseolus vulgaris*[1]. For this reason, it has been used as biofertilizer in agriculture to improve crop productivity of this leguminous that has great importance in mexicans diet[2]. At the laboratory scale, the study and propagation of the microorganism is basically realized in complexes culture media like PY and YEM, or in minimal media (MM) mainly with succinate as carbon source. However, the cell densities that are achived with MM are relatively low. The aim of this work was to reformulate the MM used by Encarnación et al. (1995)[3], evaluating different $K_2HPO_4$ concentrations in a range of 0.2 to 2.0 g/L. Moreover, we have compared the growth profiles using glucose and succinate as carbon sources. The growth was evaluated at flask scale with 100 mL of MM by determining optical density spectrophotometrically ($OD_{600nm}$) and quantifying colony-forming units (CFU/mL) by plate counts. In addition, substrate consumption and pH profiles was determined along kinetics.

The results show that increasing the concentration of $K_2HPO_4$ in MM with succinate affects growth. However, when the concentration of $K_2HPO_4$ is enhanced to 1.5 g/L in MM with glucose, the optical density reached is more of the double with respect of the original formulation with succinate. Moreover, the colony-forming units with reformulated MM with glucose are one order of magnitude greater, and the specific growth rate is approximately 40% higher than MM with succinate. The complete depletion of glucose in MM with 1.5 g/L $K_2HPO_4$ is given at 32 h of culture and shows a tendency to acidification. The culture reformulation medium could allow the microorganism characterization growth in future experiments in order to understand the metabolic response under different oxygen environmental conditions. The MM reformulated could also function as a production medium for a liquid bacterial inoculant presentation with the possible advantage of being less prone to be contaminated by limiting the nutrients concentration.

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# Cytotoxic activity on leukemia cell lines of exopolysaccharides and intrapolysaccharides from *Humphreya coffeata* (Berk) Stey.

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*Humphreya coffeata* is a mushroom used in the traditional medicine. Recent studies have demonstrated that its culture filtrates have cytotoxic and genotoxic activity on cancer cells *in vitro*. In general, many mushroom extracts of fructiferous bodies and broth culture contain several active components like intrapolysaccharides (IPS) and exopolysaccharides (EPS) with medicinal properties (antitumor, antibiotic, antioxidant, Immunomodulatory and hepatoprotective, among others). Hence, the objective of this work was the obtaining of IPS and ESP from *H. coffeata* submerged cultures. Also to test their cytotoxic activity in chronic myeloid leukemia cells (K562) and acute lymphoblastic leukemia cells (Jurkat E6-1).

The results showed that the maximum production of biomass was 8.41±0.45 g/L, EPS of 2.45±0.2 g/L and IPS of 0.089±0.01 g/g in three realized bioreactors. The infrared spectrum suggested that the EPS and IPS have the characteristic structure of sugar with pyranose configuration. EPS and IPS exhibited a cytotoxic activity in a dose-dependent manner (312-10000 µg/mL) in K562 cells at 72 h (determined by Resazurin assay). In Jurkat E6-1 cells the IPS have a cytotoxic activity in a dose-dependent manner (1250-10000 µg/mL) at 72 h. Finally we observed that the EPS have cytotoxic activity in the higher concentration evaluated (10000 µg/mL in Jurkat E6-1 cells).

This work provides the evidence of cytotoxic activity of EPS and IPS from *H. coffeata* in leukemic cells (K562, Jurkat E6-1) and allowing us to suggest that *H. coffeata* as a natural source of metabolites with a potential medical application.

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# OXYGEN CONCENTRATION IN CULTURE OF *Metarhizium anisopliae* GENERATES CONIDIA WITH SUSTAINED ANTIOXIDANT ACTIVITY

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**Keywords: Glutathione peroxidase, oxidizing atmospheres, adaptation to the environment.**

## Introduction.

*Metarhizium anisopliae* is considered an alternative for the control of agricultural pests. Aerobic organisms are subjected to reactive oxygen species (**ROS**) product of metabolism, these organisms have developed antioxidant defenses including Glutathione peroxidase enzyme [1] (**GPx**).

The aim of this study was to determine the effect of different concentrations of $O_2$ during cultures of *M. anisopliae* on Glutathione peroxidase activity (**GPxA**) in conidia.

**Methodology.** The strain *M. anisopliae* CP-OAX remained under normal atmospheric conditions (21% $O_2$) until 60 h, thereafter atmospheres were varied with concentrations of 16, 30 and 40% $O_2$ every 24 h. Conidia were harvested and disrupted, subsequently **GPxA** was measured following the instructions of a commercial Kit (Cayman Chemical). At the same time the soluble protein concentration was measured by the Bradford method, the reaction was measured at 595 nm.

**Results**. **GPxA** was measured in conidia of *M. anisopliae* obtained with different oxidizing conditions in the atmosphere of culture (16, 21, 30 y 40% $O_2$); conidia obtained with 21 % $O_2$ had an increase in **GPxA** through time, reaching a maximum peak (132 h) and then decreasing (156 h). Interestingly, conidia obtained with oxygen concentrations above normal (30 and 40% $O_2$) achieved moderate elevation of **GPxA,** only 2 to 3 times higher respectively, compared with **GPxA** measured at 24 h after the first oxidant pulse (84h culture); by contrast, under normal atmospheric conditions (21% $O_2$), **GPxA** increased up to 10 times. Conidia are preservation units that eventually germinate, these are equipped with a machinery of enzymes and proteins that allow them to interrupt latency; this is a shift to a respiratory metabolism, associated with the formation of **ERO**[2]. Thus, conidia are provided with **GPx** able to use $H_2O_2$ as a substrate to oxidize the **GSH**, our results suggest that conidia harvested from more oxidizing conditions (>21% $O_2$) are adapted to oxidizing environments, acquiring high antioxidant capacity (up to 4 times) from the beginning of conidiation (84 h), and conidia of successive times, have a sustained enzyme **GPx** activity, which does not change in the last points of the kinetic ($p<0.05$, uppercase letters). This has practical implications since if all conidia produced in a batch have a sustained antioxidant capacity, they could have better opportunities to survive and infect insect hosts.

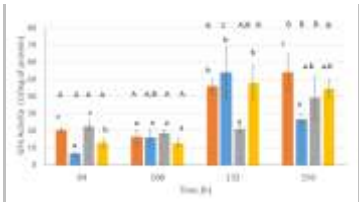

**Fig. 1**. **GPx** activity in conidia of *M. anisopliae*, obtained with different concentrations of oxygen in the atmosphere (16% ■, 21% ■, 30% ■ y 40% ■). Bars with different *lowercase letters* show significant differences between treatments, bars with different uppercase letters show significant differences through time to the same treatment (Tukey's test $p<0.05$).

When the enzymatic activity of **GPx** decreased in fungus *Alternaria alternata*, the fungus was not able to infect its host [3]; in contrast, the conidia obtained with atmospheres 21% and 30% $O_2$ are able to kill up to 90 % of larvae *Tenebrio molitor* [4]. On the other hand, conidia obtained under hypoxic conditions (16% $O_2$), also generated **ROS** [5]**,** since the last electrons acceptor is not present, our results suggest, that although **GPxA** increased 2.6 times at 156 h, these conidia deal with a possible an endogenous stress that does not allow them to kill the insect host.[4]

**Conclusion**. Atmospheric modification with a high concentration of $O_2$ generate conidia of *M. anisopliae* with more uniform antioxidant capacity

**Acknowledgments.** The authors thanks to CONACyT for the financial support.

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# Molecular chaperone ZmFES1a: cisgenic over-expression to improve thermo tolerance.

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In plants tolerance to stress conditions, particularly to high temperature, involves a complex response. Heat shock proteins (Hsp) are fundamental part of this response and in the acquisition of thermo tolerance. In maize, the ortholog of the human Hsp70-binding protein, ZmFES1a, has been described and its role on thermo tolerance development has been suggested. Considering the global warming and climate change scenario, that we are facing, the Improvement of plants with tolerance to high temperature is a key subject. Recently gene modification by cisgenic constructions is a biotechnological method, to improve agronomic traits. Thus avoiding the use of transgenes is a safer environmental approach that maintains the original cultivar characteristics and allows genetic improvement of crops. In this research we achieve the over-expression of ZmFES1a in maize plants by biobalistic insertion of a maize cisgenic construction. This comprised the ubiquitin promoter, cDNA of ZmFES1a and the terminator of Rubisco small sub-unit, these elements considered only maize DNA sequences. Embryogenic callus of Tuxpeño race was bombarded with the cisgenic construction and the regenerated plants were grown under green house conditions until the reproductive phase. Evidence demonstrated that the cisgenic construction was integrated to the plants genome and protein was over-expressed. Further analysis on thermo tolerance acquisition was performed. We consider that the cisgenesis improvement technology is a promising tool that needs further development for crops improvement, to face emergent climate issues.

Acknowledgments. This project was supported by grants CONACYT: 213872 and 252001. Also PAIP grant 5000-9123.

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# Autoreplicative plasmids for genetic engineering in *Lachancea kluyveri*

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In recent years, the budding yeast *Lachancea kluyveri* has gained importance as a model organism. In this yeast has been studied the synthesis and degradation of branched-chain amino acids, nucleic acid precursors degradation, characterization of mitochondrial respiratory-deficient strains, petite mutants, crabtree effect, ethanol production and anaerobic respiration. Traditionally, *Sacharomyces cerevisiae*, has been widely used as a model organism and many methods for *S. cerevisiae* are applicable to *L. kluyveri*. However, efficient tools for the systematic study of genes in *Lachancea kluyveri* are missing. In this work, we present the construction of stable autoreplicative episomic plasmids suitable for mutant complementation, gene expression studies or genetic screen in *L. kluyveri*. The constructed plasmids contain the selectable marker gene *URA3* that provide a convenient tool for genetic manipulation in this non-conventional yeast.

Keywords: Autoreplicative plasmids, yeast.

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# Structural characterization of bacteriophage M13 in acid pH values.

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The bacteriophage M13 has been modified, at the N terminus of each copy of pVIII major coat protein, with a fused peptide with affinity for a specific target, which allows its use as a versatile scaffold for the design of nanoarchitectured structures and materials. As starting point to study the interaction precursor/peptide in the biomineralization reactions it is important to characterize the stability of the phage M13 in an acid environment, because some of the reactions take place in these conditions. Williams *et al.* reported phage-M13 pVIII undergoes a conformational change to a β-sheet conformation at high temperatures, as detected by circular dichroism spectroscopy (CD). Stopar *et al.* published that the major coat protein can change its conformation to accommodate three distinctly different environments: phage filamentous, S-form, and membrane-bound form, all characterized by CD. In this work, we report phage M13 conformational changes that occur in an acid environment, detected with fluorescence and far-UV CD spectroscopy. The phage M13 was incubated in solutions with pH 2.81 and 3.69 during a period of time and then it was resuspended in a neutral solution for analysis, because the phage precipitates at that pH conditions. We used intact and sonicated samples, pH 7.3, as controls.

The results of fluorescence showed that the after 1260-min incubation at pH 2.81 the spectral mass center increases only two units with respect to the intact phage. The sonicated sample showed a similar shift. These results indicate a little increase in the exposure of tryptophan to the aqueous phase. In the CD study, we observed that samples subjected to acid environments for 120 min showed a slight decrease in the intensity of the bands at 208 and 222 nm, but the quotient $[\theta]_{208}/[\theta]_{222}$ kept the value of the intact sample. Nevertheless the sonicated sample showed a β-sheet-like spectrum (see Figure 1).

In conclusion, the phage M13 is stable in the studied conditions at least for 120 minutes, and, therefore, it would be able to perform biomineralization reactions.

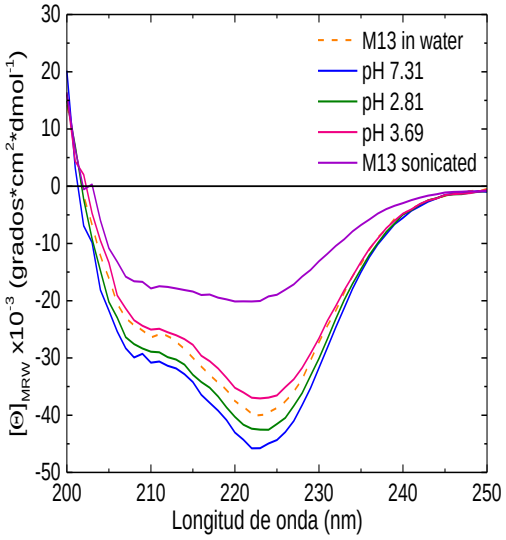


***Figure 1:****Dichroism spectra of the sample of phage M13 in different conditions.*

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# Laccase activity and specific growth rate of *Pleurotus ostreatus* NRRL 3526 grown on solid-sate fermentation and submerged fermentation

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Laccases are glycoproteins that catalyze the oxidation, methylation, demethylation, depolymerization and polymerization of phenolic compounds (1). These enzymes have been found in white-rot fungi, insects, plants and bacteria (2). *Pleurotus* is an edible fungus able to produce such enzymes. It has been suggested that the number and type of isoenzymes depend on the fungus growth conditions (3). In this work, the specific growth rate and the laccase activity were evaluated in *Pleurotus ostreatus* NRRL 3526 grown in solid-state fermentation (SSF) and in submerged fermentation (SmF). Mycelial fragments (4 mm diam) taken from the periphery of a 7 days old colony were used as inoculum in all the experiments. SSF studies were carried out in Erlenmeyer flask (250 ml) containing 0.5 g of polyurethane foam as inert support added with 15 ml of culture medium. SmF analyses were undertaken in Erlenmeyer flask of 125 ml containing 50 ml of culture medium. Samples were taken each 24 h for 22 days (stationary phase). In all the samples, mycelial biomass was obtained by filtration and the specific growth rate was evaluated (μ) using the logistic equation as previously reported (3). Intracellular laccase activities were evaluated in the supernatant using 2,6-dimethoxyphenol as substrate. Values of maximal biomass ($X_{max}$) were 5.9 and 7.3 g/l and and μ values were 0.055 and 0.02 $h^{-1}$ for SSF and SmF, respectively. Highest laccase activities were 8400 and 17500 U/l for SSF and SmF, respectively. These results suggest that the highest laccase activity was observed in the exponential phase of the fungus in both fermentation systems.

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# Promoter expression pchiA endochitinase chiA74 compared with two dependent promoters sporulation in *Bacillus thuringiensis*

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*Bacillus thuringiensis* is the most widely biological insecticide used to control insect plagues in agriculture. It is a sporogenic bacterium of agroindustrial interest, gram-positive, cosmopolitan, which main characteristic is the production of crystals formed by Cry proteins. Cry proteins synthesis is associated with the sporulation process. Chitinases are enzymes that hydrolyze chitin and may have various uses, including a synergistic effect with the Cry proteins. In our group we have worked for several years with the ChiA74 endochitinase, which is synthesized by a Mexican strain of *B. thuringiensis*. The aim of this study was to evaluate the time in which the expression of wild pchiA promoter takes place, and compare it with two sporulation dependent promoters of *B. thuringiensis*, the pcytA and pBtI-BtII. The first promoter controlling expression of the ChiA74 endochitinase, and the last two, controlling Cyt1A and Cry1 proteins expression. Three constructs were made (pHT3101-pchiA-gfp, pHT3101-Bti, BtII-gfp, pHT3101-pcyta-gfp) in which the GFP gene was used as reporter; the GFP gene is under the regulation of pchiA, pcytA and pBtll-Btll promoters. An acrystalliferous strain of *B. thuringiensis* was transformed with the constructions (pHT3101-pchiA-gfp, pHT3101-Bti, BtII-gfp, pHT3101-pcyta-gfp) and analysis was carried out by quantitative PCR. In the poster we present comparative data related to the time of expression of the three genes.

**Keywords**: *Bacillus thuringiensis*, chitinase, Cry proteins, regulation.

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# The importance of pH variation in different growth culture media during the production of recombinant proteins in *Escherichia coli*

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*Escherichia coli* is a commonly used organism for heterologous protein production because it offers advantages such as fast growth rate, easy plasmid transformation, low-cost culture media, protein yields (up to 20 g/L), and can overcome different stressful conditions that could arise during a bioprocess. For instance, *E coli* is able to support extreme pH values occurring along the human digestive tract, as in the stomach (pH 1-3) and small intestine where pH values may reach 8. Extracellular pH may modify to the growth of microorganisms, while intracellular pH affects the enzymatic properties, protein stability, structure of nucleic acids and many other biological molecules. Stress responses to pH alterations in *E. coli* are directed to maintain intracellular pH within physiological values (7.6 ph). When this microorganism is cultured in the laboratory with sufficient nutrients, under controlled temperature, pH and oxygen levels, optimal maximum growth rate will be achieved and the best yield. However, during bioprocesses development and characterization, shake flasks are commonly used, where pH and oxygen concentration change. Particularly, it has been observed that complex media increases pH level during the stationary phase, this increase in pH is a common effect in peptone based media; both the LB and SB media is mainly composed of nitrogen-containing complex compounds and cells are forced to use these as a source of energy. Whereas, in salt-based media where the carbon source is glucose pH decreases by the production of acetate and other organic acid.

Our aim in this study, was to assess how culture media affects the growth of a recombinant *E. coli*, and how this affects the production of a recombinant protein model. In the search for the best strategy for the production of heterologous protein, we grew the bacteria under different culture media Luria Bertani (LB), Super Broth (SB) and minimal medium (MM). The kinetic parameters (growth rate, doubling time) and stoichiometric parameters (specific productivity) were evaluated. Until now we have found higher growth (around 20-30% more biomass) and better final recombinant protein concentration (approximately 50% higher) in LB and SB media, compared with the MM. This may be due to the fact that during growth on rich medium such as LB and SB, cells do not need to synthesize most of the precursor molecules (like amino acids) because they are already present in the medium. The perspective is to evaluate the characteristics of soluble proteins and protein aggregates when produced in different conditions.

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# Monitoring of volatile organic compounds (VOCs) during plant-microorganism interaction in real time.

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Plants play a crucial role for other organism's survival, with functions such as food supply and oxygen production. For humans, plants additionally have economical importance, because plants provide raw materials in agroindustries, energy production and the manufacturing of diverse products.

Biotechnological research aims to increase the productivity of food plants, to optimize plant defense, and to employ beneficial interactions with other plants and microorganisms. For these studies, the characterization of the plant metabolism is of great interest.

The profile of Volatile Organic Compounds (VOC's) changes during its interaction with microorganism [1]. However, with actual technology it is difficult to elucidate the exact moment, in which molecules are released.

To determine the biological relevance of specific molecules, the temporal information has to be taken in account. Therefore, we developed an automatized system for the online monitoring of VOC profiles or specific metabolites of interest. Our analyzer provides different interfaces for the ambient analysis of metabolites, as well as a residual gas analyzer (RGA). With this method we will be able to get a closer view on the chemical level of microorganism-plant interactions, providing complementary information to the already established biochemical pathways [2].

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# Cloning and expression of recombinant J1-1 defensin from *Capsicum chinense* in *Escherichia coli*

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Defesins are part of the innate immune system of terrestrial plants. They play a role as the first line of defense in the organism. Some plant defensins characterized from Solanaceous have strong antifungal activities, and several modes of actions (MOA) have been elucidated. This MOA are related to the interaction with membrane and cell wall constituents. The most studied solanaceous plant defensins, NaD1 and TPP3, show interactions with phosphatidylinositol (4,5) bisphosphate ($PIP_2$) *in vitro*. On the other hand, class I defensins from *Medicago truncatula* and *Medicago sativa*, MtDEF4 and MsDEF1, are capable to interact with phospholipids as the Solanaceous defensins, although for MtDEF4, show interactions preferably with phosphatidic acid (PA), a second messenger involved in a variety of plant functions and processes.

In the present work, we have cloned and expressed the gene for a point mutant (J1-1/K45E) of the plant defensin J1-1 from *Capsicum chinense*. J1-1 is a peptide of 47 amino acids, a class I defensin reported with antifungal activity against *Fusarium oxysporum* and expressed exclusively in mature fruits (Meyer et al.,1996). The presence of the fusion peptide of J1-1_K45E was confirmed in the bacterial extracts by western blot. J1-1/K45E which is found in the insoluble fraction of the *E. coli* extracts, was purified by affinity chromatography and its phospholipid binding profile will be determined *in vitro*.

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# Metabolic engineering for increasing the production of rhamnolipids in a *Pseudomonas aeruginosa* strain

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**Keywords:** *Pseudomonas aeruginosa*, rhamnolipids, polyhydroxyalkanoates.

**Introduction.** *Pseudomonas aeruginosa* is an ubiquitous Gram-negative Gamma-proteobacteria capable of producing a large variety of metabolites, enzymes and polymers – some of these with potential biotechnological application – that allow the colonization of multiple microhabitats. *P. aeruginosa* produces – at its late logarithmic phase of growth – a biosurfactant known as rhamnolipid (RL), which is a glycolipid composed of a hydrophobic fatty acid moiety and a hydrophilic moiety of one or two molecules of rhamnose. The carbon source most widely used in industrial processes is carbohydrates. In this scenario, the hydrophobic moiety is provided by the fatty acid *de novo* biosynthesis pathway FASII. β-hydroxyacyl-ACP – which is an intermediate in fatty acid biosynthesis – is dimerized by the RhlA enzyme to produce β-D-(β-D-hydroxyalkanoyloxy)alkanoic acids (HAA), which is an important precursor of RL. However, β-hydroxyacyl-ACP can also be transacylated by PhaG enzyme to produce β-hydroxyacyl-CoA, which is the precursor of the biopolymer polyhydroxyalkanoate (PHA) biosynthesis. The objective of this work is to increase RL production in a *P. aeruginosa* strain by eliminating the competence of β-hydroxyacyl-ACP between the synthesis of this biosurfactant and PHA biosynthesis.

**Methods.** In order to block PHA biosynthesis pathway, a *P. aeruginosa* strain with three knockout mutations on *phaG*, *phaC1* and *phaC2* chromosomal genes was generated. These mutations were constructed by using the lambda Red recombinase system reported by Lesic & Rahme, 2008[1]. This *phaG⁻ phaC1⁻ phaC2⁻* triple mutant was transformed with a plasmid construction in order to overexpress *in trans* either *rhlAB* or *rhlABR* operons. *P. aeruginosa* strains were cultivated in PPGAS[2] medium. RL were extracted and quantified using the orcinol assay. PHA was quantified by acid hydrolysis and crotonic acid.

## Results

| Strain | Ramnose in RL (μg/mL) | PHA* (μg/mL) |
|---|---|---|
| PA14 | 163.63 ± 3.75 | 4.73 ± 0.63 |
| PA14 *phaC1⁻ phaC2⁻ phaG⁻* | 175.62 ± 6.27 | ND |
| PA14 *phaC1⁻ phaC2⁻ phaG⁻* + pHERD20T/*rhlAB*** | 223.90 ± 12.15 | ND |
| PA14 *phaC1⁻ phaC2⁻ phaG⁻* + pUCP20/*rhlAB**** | 190.78 ± 3.60 | ND |
| PA14 *phaC1⁻ phaC2⁻ phaG⁻* + pUCP20/*rhlABR**** | 245.41 ± 10.57 | ND |

* ND: Not Detected
** Induced with 0.2% arabinose
*** Under its own promoter

## Conclusions

- The absence of the PHA biosynthesis pathway – by itself – does not increase RL production.
- RL production increases by the overexpression of *rhlAB* and *rhlABR* operons.
- The major increase of RL production was obtained by *rhlABR* operon overexpression.

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# Biological effects of cyclodipeptides from *Pseudomonas aeruginosa* PAO1 in human cancer cells

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*Pseudomonas aeruginosa* is an opportunistic pathogenic bacterium of plants and animals, which produces virulence factors to infect and colonize the host. Recently a new kind of bacterial molecules that act as virulence factors have been identified, these are known as cyclodipeptides (CDPs). Additionally, these are considered as a novel class of small molecules synthesized by microorganisms, and which possess biological activities such as anti-cancer, anti-fungal, and antibiotic. In this work, we describe that the mixture of the CDPs produced by *P. aeruginosa* cyclo(L-Pro-L-Tyr), cyclo(L-Pro-L-Val) and cyclo(L-Pro-L-Phe) exhibit cytotoxic properties in the HeLa and CaCo-2 cancer cells lines, whose inhibition of cell proliferation involves an apoptotic pathway. Results showed that the fractions of the CDPs produced by *P. aeruginosa* purified by HPLC were capable of induce in same levels apoptosis in HeLa and CaCo-2 lines, with a dose-dependent manner, while the CDPs analogs and with synthetic origin showed less cytotoxic effects on these cancer cell lines. The findings indicate that the effect produced by the purified CDPs and its mixture showed increased cytotoxic activity than the synthetic CDPs, suggesting an effect associated with their stereochemical properties of the bacterial origin with its biological activity. Additionally, the results indicate that the CDPs mix was more active for induction of apoptosis in the HeLa and CaCo-2 lines than the isolated compounds. Finally, this study suggest that the CDPs could be considered as a potential anti-cancer molecules.

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# Caracterization of composts made from organic waste of Tepetitla de Lardizabal, Tlaxcala.

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*Keywords:* Compost, vermicompost, solid urban waste.

**Introduction.**

The decrease in the generation of solid urban waste (SUW), processing and arrangement thereof, is one of the bigger ecological and health challenges. In Mexico annually 41 million tons of RSU are generated, at least 47% is organic waste (SEMARNAT, 2012). In the state of Tlaxcala 80% of SUW ends up in the landfill and 2% in open dumpsites (INECC, 2012), this polluting soil, air and water due to deficiency in the process of final disposal.

In this work we characterized the physico-chemical properties of composts and vermicompost, elaborated with SUW and farming waste from Tepetitla, to evaluate its potential use in the fertilization of country lands.

**Methodology.**

Four composts were produced by the method of piles with compositions: 100% cow manure (E); 100% vegetable waste (RV); 20% plant waste and 80% manure (RV/E) and 100% corn stover (RA). Weekly piles were irrigated and turn over; the process lasted 4 months. Vermicompost (VE) was prepared in a drawer with 100% manure, using *Eisenia fetida*. At the end of composting it was measured pH, electrical conductivity (EC), moisture and germination index (GI), based on Zucconi's methodology (1981). It was quantified organic C, P and K.

**Results.**

With all the wastes was possible to obtain mature composts, which were compared with the NOM-021-SEMARNAT-2000 and NADP-020-AMBT-2011. Agreed to the regulation of composts, they are strongly alkaline in all cases, the CE <4.1 dS/m, the % moisture is between 10.1-2,9%, optimal for composts type B (organic farming and reforestation use). According to the IG, RA is suitable for nurseries and VE for organic farming because they aren't phytotoxic. The other composts conforming to the IG can be used in urban green areas and reforestation. All treatments are high in organic C, K and P.

Table 1 shows the results, which are similar to other reports where use USW, farming waste and manures, getting final products that worked as fertilizers and increased vegetable production (Jara et al. 2015 and Bautista et al. 2014).

| Compost | pH | CE dS/m | IG | % Organic C | K (%K2O) | P (mg/kg) | % Moisture |
|---|---|---|---|---|---|---|---|
| RV | 9.0±0.02 | 3.4±0.00 | 51.5±10.11 | 28.53% | 0.84 | 320 | 2.9±0.45 |
| E | 9.5±0.02 | 4.1±0.00 | 63.4±28.99 | 47.06% | 1.4 | 254 | 6.9±0.73 |
| RV/E | 9.3±0.02 | 3.8±0.00 | 64.9±2.12 | 45.11% | 1.41 | 590 | 4.2±0.19 |
| RA | 8.5±0.01 | 2.3±0.00 | 89.8±1.9 | 54.21% | 1.14 | 106 | 10.1±0.48 |
| VE | 9.5±0.00 | 2.2±0.00 | 79.8±6.7 | 27.23% | 0.81 | 264 | 4.9±0.52 |

Table 1. Properties of compost and vermicompost.

**Conclusions.**

The properties of the compost obtained depend on the type of waste used. In this research we started from alkaline wastes by this reason resulting alkaline products. Taking into account the parameters stipulated in the regulation, RA and VE achieve all criteria (except pH) to be use in country lands as fertilizers, taking care of used them in an appropriate ratio

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# Immunogenic properties of ectodomain of Porcine rubulavirus Hemagglutinin-Neuraminidase produced in the yeast Pichia pastoris

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The viral blue-eye disease of pigs (EOA) was detected in porcine farms of La Piedad, Michoacán, México in 1980. The etiological agent is a *paramyxovirus* classified like *Rubulavirus porcine* (**RVP**), which causes neurological problems, reproductive, respiratory and opacity corneal in 1-10% of the cases. The Hemagglutinin-Neuraminidase (**HN**) plays an important role for the RVP´s biological cycle and is the protein the most immunogenic of virus. In the Hemagglutinin-Neuraminidase's ectodomain (**eHN**) we found the immunogenic sites, glycosylation, active site and is the most interesting for develop a vaccine.

We report in this study the obtaining of eHN of RVP-HN through the Pichia pastoris expression system. The eHN open reading frame (ORF) was amplified from the PAC1 strain of by PCR, then, was subcloned into pPICZαB-eHN expression vector. The vector was integrated into the AOX1 promoter by displacement in the yeast chromosome and phenotype Mut+ was obtained in the transformants. The strain was named X33-eHN and was carried to expression using methanol as unique carbon source and inducing in high cell densities. The eHN expressed into P. pastoris X33-eHN was recovered from cell-free medium, featuring up to 67 nmol/min/mg after 6 days of expression. The eHN was recognized by the serum of infected pigs with strains currently circulating in the Mexican Bajio region by western blot and ELISA test. The protein was purified by one step of affinity chromatography (IMAC) with 92% yield and recovered 1.2 mg of purified protein by each liter of culture. The eHN induces antibodies in mice strain CD-1, after immunization during 28 days with specific recognition in ELISA test. These antibodies were able to inhibit replication more than 80% by viral neutralization assays in cell culture.

The project generated a protein eHN with similar characteristics to the native HN and which may be a good candidate to propose a vaccine or to use the antigen in a serological diagnostic test.

- José Luis Cerriteño-Sáncheza, Gerardo Santos-López, Nora Hilda Rosas-Murrieta, Julio Reyes-Leyva, Sandra Cuevas-Romero, Irma Herrera-Camacho. Production of an enzymatically active and immunogenic form of ectodomain of Porcine rubulavirus hemagglutinin-neuraminidase in the yeast Pichia pastoris. Journal of Biotechnology 223 (2016) 52–61

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# Critical path for obtaining nutraceuticals species of Rubus spp . , and their relationship in preventing metabolic diseases.

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**Key Word:** (Nutraceutics, *Rubus spp.* Metabolic Diseases.)

Abstract

Mexico is positioned as an emerging economy, where the population presents with public health problems arising from the implementation of this economic model, large amounts of calories and low quality nutritional, and there are economic niches that set standards worldwide in production and distribution of food, but with an impact on regional economies and agricultural societies, where Mexico has changed since the twentieth century to the current century (from an agricultural society to a society steeped in world markets, with the opening and release of sectors including food processing, signing free trade agreements). In this work the roadmap that has been implemented through the linkage between Universidad Michoacana de San Nicolás de Hidalgo and producers' cooperative Zarzamich described., Of Ziracuaretiro, which has helped develop the metodolgías they have achieved and characterized chemically, to the nutritional compounds with high added value in the food industry (nutraceuticals), derived from the fruits of Rubus spp genera., that do not meet export quality. Allowing generate data that enable diversification in production not only of a primary industry also can pose a nascent mining industry nutraceuticals as fatty acids polyunsaturated type, oleic, linoleic and linolenic acid, which can be exploited in the pharmaceutical industry for the prevention of degenerative diseases, as part of comprehensive treatment, all this as a strategic part in the production and processing of red fruits produced in Michoacan.

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# Recombinant overexpression of trypsin III mutants from Monterey sardine (*Sardinops sagax caerulea*)

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Monterey Sardine (*Sardinops sagax caerulea*) is the main fishery product in Mexico, the most part of them is destined for the processing of products that generate waste. Viscera are by-products advantage for obtaining proteolytic enzymes as trypsin. Obtaining fish trypsin is of scientific importance to study the structure and function relationship in cold adaptation, as has been shown to exhibit higher catalytic activity at low temperatures than their mesophilic counterparts. In order to understand the function of certain amino acids leading to the cold adaptation, this aim of this study is to evaluate different conditions to obtain higher yield of recombinant trypsin mutants in *Escherichia coli*, in order to probe the relationship between structure and function in trypsin III mutants. We used different strains of *E. coli* BL21 (DE3): Gold and origami, they were transformed with plasmids containing trypsin III of Monterey sardine in its mutant forms A233N and L234Y cloned in a pET-32a vector, which produces a thioredoxin fusion protein. Overexpression was analyzed by SDS-PAGE, enzymatic activity with substrate BapNA and zymography. Trypsin III mutants were successfully obtained recombinantly. Our evidence determined that the strain of *E. coli* BL21 (DE3) Gold had better yield of soluble protein and higher trypsin activity.

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# Induction of *in vitro* morphogenetic response of *Kalanchoe daigremontiana* for the production of secondary metabolites.

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*Kalanchoe daigremontiana* is a succulent plant used as ornamental. It presents diverse medicinal properties because of its secondary metabolites. Some of these metabolites are the bufadienolides that have cardiotonic and cytotoxic properties, so their production are very important for the medical area. The propagation of *K. daigremontiana* using *in vitro* culture is a good alternative to improve the yield of these metabolites. Temporary immersion systems (TIS) provide discontinuous contact with the culture media, improving the transference of nutrients, renewing the gas phase and reducing cost because agar is not needed in this system. In the present work, *K. daigremontiana* was *in vitro* established using multiple disinfection protocols based in the use of ethanol (70%) and different concentration of sodium hypochlorite (0.3, 0.5 and 0.75 %). Once the *in vitro* plants were established, explants were taken from leaves and seedlings. The explants were grown in semisolid media and temporary immersion systems supplemented with 1 mg $L^{-1}$ Benzylaminopurine and several levels (0 0.5, 1, 1.5 mg $L^{-1}$) of 2,4-Dichlorophenoxyacetic acid, for callus induction. The measured variables were number of leaves, plant height, timing of callus induction and percentage of induced calli. A faster callus-growing biomass was observed using the TIS. Likewise, callus appeared on explants in a shorter time when TIS was used. The presence of secondary metabolites was detected through colorimetric assays and thin layer chromatography (TLC). This work opens the possibility to use TIS as an efficient system for the production of *Kalanchoe daigremontiana* secondary metabolites.

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# *In vitro* micropropagation of *Echinocactus grusonii* (Golden Barrel Cactus)

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*Echinocactus grusonii* (Golden barrel cactus) is an endemic cactus to Mexico, and is located in diverse zones, which includes Queretaro, Zacatecas and Hidalgo. This cactus has great biological, cultural and ornamental importance. In recent years, it has occurred a drastic decrease in its natural populations, due to practice of illegal recollection and loss of its habitat, therefore, it has been cataloged as extinction-threatened by the NOM-ECOL-059-2001, and like critical-endangered species by the UICN-2004. The objective of the present work was to establish a methodology for the efficient *E. grusonii*-micropropagation into a temporal immersion system. Seeds of this cactus were disinfected using $H_2SO_4$, TWEEN 20, 70 % ethanol and NaClO. Several treatments were evaluated using different plant-growth regulators and immersion times into MS liquid medium (Murashige and Skoog, 1962). After 6 months of cultivation, the treatment with 4 mg/L of Benciladenine (BA) and 0.5 mg/L of Indoleacetic acid (IAA), with an immersion time of 1 minute every 72 hours, generated the greatest number of buds (47 buds/explant). Nonetheless, bud abnormalities were observed. Taking into consideration these results, hormonal pulses (5, 10, 20, and 30 min) using BA (40 mg/L) and IAA (5 mg/L), and immersion time of 1 min every 72 hours, were carried out. Under these conditions, the explants of *E. grusonii* were higher than control explants. The micropropagated plants were hardened in enriched substrates. This methodology would help to replant and recover natural *E. grusonii*-zones where this species has been lost.

**Keywords: Cactus, plant tissue cultures, pulses, SIT**

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# Determination of laccase and peroxidase activities of the moderate halophile *Aspergillus caesiellus* growing on phenanthrene and benzo(a)pyrene as only source of carbon.

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Polycyclic aromatic hydrocarbons (PAHs) are xenobiotics that persist in the ecosystems during long periods of time, due to its water low solubility. Some PAHs are mutagenic and carcinogenic, highlighting the importance of their removal from the environment. Some microorganisms, such as bacteria, algae and fungi are capable of metabolize PAHs. Particularly, ligninolytic fungi are able to degrade those compounds due to structural similarity with lignin (a polymer formed by units of phenolic acids) using enzymes such as peroxidases and laccases. Anthropogenic activities such as oil spillages in the sea or production of industrial waste water are a challenge for bioremediation due to the high salinity. The studies have focused in the characterization of PAHs removal by microorganisms growing in the absence of salt. Our laboratory has isolated a moderate halophile fungus, identified as *A. caesiellus* by polyphasic criteria. This fungus is capable to grow on solid state fermentation of lignocellulosic substrates such as sugarcane bagasse, wheat straw and cornstalks among others. Also, there is presence of manganase peroxidase activity, one of the ligninolytic activities. The aim of this work is to determine the activities of peroxidases and laccases of *A. caesiellus* when is growing on the presence of phenantrene or benzo(a)pyrene as the only source of carbon under halophilic conditions.

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# Evaluation of benzimidazoles as an alternative in staining of nucleic acids in agarose gel electrophoresis

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The nucleic acids staining in agarose gel is one of the most commonly used methodologies in molecular biology laboratories, where assays based on the capacity of some compounds to fluoresce when they are irradiated with UV light are made. For several years, ethidium bromide was the only chemical compound used for visualize nucleic acids by staining. Nevertheless, this is a compound considered mutagenic due to the way it bonds to the nucleic acids since it intercalates into double helix of the DNA. In the last decade, they were launched two SYBR® compounds that can be used like ethidium bromide with the same results. More recently, it has been demonstrated that some benzimidazoles, as well as molecules with similar structures, have the property of emitting light in the visible spectrum when they are irradiated with UV light and binding to the DNA with no need of intercalate.

Starting from this, we studied the effect of eight benzimidazoles; four of these having a 2-(4-substituted aryl) imidazo[1,2-*a*]pyridine moiety (4-substituents used were bromine, fluorine, methyl and methoxyl) and other four having a 6-(4-substituted aryl) imidazo[2,1-*b*]thiazole moiety (4-substituents used were bromine, fluorine, methyl and hydrogen). The maximum excitation of fluorescence of the eight compounds was determined to be in the UV spectrum obtaining the highest point at 325 nm both for imidazopyridines and imidazothiazoles.

The binding ability with nucleic acids in agarose gels was analyzed, obtaining that seven of the eight compounds, at concentrations between 0.25 and 1.25 µg/ml, were able to make visible DNA concentrations of 400 ng. Among the seven compounds, imidazopyridine-fluorine and imidazopyridine-methyl stood out, making visible DNA concentrations up to 20 and 10 ng, respectively. The results obtained suggest that these chemical compounds are a real alternative to the use of ethidium bromide for staining nucleic acids in agarose gel electrophoresis assay.

In subsequent assays, toxicity, reverse mutations and effect in efficiency of cloning of these compounds are going to be analyzed.

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# Protein engineering for molecular recognition; the cysteine protease inhibitor 1 of *Entamoeba histolytica*.

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Protein-protein interactions (PPIs) are pivotal for most cellular processes. The ability to block or promote such PPIs is inherent to medical therapies and biotechnological applications. During the last years, through protein engineering techniques, mostly by random generation, a reduced number of proteins have been used as scaffolds for protein design, including: the fibronectin type III domain, the B domain of protein A, PDZ, SH3, the ankyrin repeat domain, T-cell receptors and antibodies, among others. Antibodies are naturally engineered proteins, historically used as high-specificity reagents for many molecular recognition applications. However, antibodies are large multi-chain molecules above 100 kDa, with disulfide cross-links and highly thermosensitive. Furthermore, they cannot be easily expressed to high levels in bacteria. These properties hamper their use in diagnostic and molecular medicine.

Although proteins are marginally stable, they can tolerate multiple amino acid substitutions and maintain their native structure. In a seminal work Colas and coworkers designed new loops in *Escherichia coli* thioredoxin to "mimic the recognition function of the complementarity-determining regions of immunoglobulins" and dubbed these engineered proteins as "peptide aptamers" (PAs). Numerous works use diverse PAs scaffolds to introduce peptide sequences typically from 5 to 20 amino acids. The desired characteristics of a PA scaffold are: tolerantion to amino acid substitutions, thermostability, abundant expression in bacteria, non toxic. The goal of a PA scaffolds is to mimic a PPI through a double constrained sequence. Just few examples of proteins with an immunoglobulin-like (Ig-like) fold as aptamer scaffolds exist, although the Ig-like fold is one of the most common structural motifs in nature.

In this work, we propose a new protein scaffold for molecular recognition, the cysteine protease inhibitor 1 of *Entamoeba histolytica* (EhICP1) which shares this Ig-like structural motif. Our crystallographic results demonstrate the capacity of EhICP1 to accept new residues within its sequence without altering its fold and also guide us to understand how EhICP1 interacts with proteases in amoeba.

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# Study of the effect of ammonium on the expression of genes coding for regulators sRNAs of Rsm Family in *Azotobacter vinelandii.*

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*Azotobacter vinelandii* is a free-living γ-proteobacteria found in soil, gram negative and belongs to the Pseudomonadaceae family also, it is an obligate aerobic microorganism. The relevance of its study is focused in the capacity of the bacteria to yield polyhydroxybutyrate (PHB) and alginates, both of them polymers with biotechnological applications. [1] Likewise, this bacterium is capable of fixing nitrogen in aerobic conditions. Function that carries out through complex metalloenzymes known as nitrogenases, which can contain 3 different cofactors for its function, Iron (Fe-Fe), Molybdenum (Fe-Mo) and Vanadium (Fe-V); unlike other microorganisms that fix nitrogen and only possess the classical Fe - Fe nitrogenase. [2]

Regulation of genes necessary for the production of PHB and alginates is carried out by posttranscriptional regulation system Rsm, consisting of several noncoding RNAs that antagonize the function of the repressor of translation protein: RsmA. The genes that coding for the sRNAs are the only direct targets of the two-component system GacS/A in *A. vinelandii*, so if we study the conditions that alter the expression of these genes, we could study the conditions or signals responds the GacS/A system.[1,3]

Exploring these conditions in the laboratory, it was found that sRNAs respond to the presence of $NH_4^+$ in the growth medium (unpublished data). To determine whether the $NH_4^+$ is a sign or has metabolic effect in the rsm sRNA expression, an insertional mutation was performed into the *nifL* gene, which belongs to a cluster of genes related to nitrogen fixation in several bacteria. Such gene encodes a transcriptional repressor protein of another gene of the cluster: *nifA*, which is a transcription activator protein of the gene encoding the enzyme nitrogenase (*nif H*). The mutant will allow us to study the expression of the sRNAs of the Rsm family in the presence of ammonia in a genetic background in which nitrogen fixation is always active. [2,4,5] The *nifL* mutant was characterized through alginate production and proceeded to study $N_2$ fixation in the presence of $NH_4^+$ by the indophenol method [5] and by RT-PCR of the *nifH* gene.

The results denote that the NifL mutant in *A. vinelandii* produces a slightly higher amount of alginate, compared to wild type (E) strain. Also, a larger presence of ammonia in the medium was also observed, compared with the wild type strain. With respect to *nifH* gene, both strains (E and the NifL mutant) in were no added $NH_4^+$ was present in the media, the expression of its mRNA was not affected. However, when adding 35mM of $NH_4^+$ in the medium, the expression it is suppressed almost entirely in the strain E, while in the mutant it is visible the expression of the messenger RNA and hence, nitrogen fixation. Likewise, the expression of sRNAs and the effect of $NH_4^+$ will be measured in transcriptional fusions which underwent the same mutation in NifL.

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# Analysis of the structural domains of ChiA74 chitin *Bacillus thuringiensis*

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*Bacillus thuringiensis* is a bacterium with insecticidal activity towards Lepidoptera, Coleoptera and mosquitoes of importance in agriculture and public health. Besides producing insecticidal proteins, also can produce extracellular macromolecules including the VIP proteins, chitinases, proteases and lipases. Various studies have demonstrated the importance of chitinase as synergistic elements of Cry proteins, mainly against Lepidoptera. However, its application is limited because most bacterial chitinases from *B. thuringiensis* have optimal activities at slightly acidic pH. In addition they are secretory proteins that need to be obtained from the supernatants and then mixed with Cry proteins to carry out bioassays. Although there are reports regarding the regulation of expression and three-dimensional (3D) structure of Cry protein, there is a lack of this type of information about chitinases synthesized by *B. thuringiensis*. In this project, our objective is to analyze the domains of ChiA74 endochitinase for subsequent crystallization. Here we report the cloning and amplification of the structural domains of ChiA74 endochitinase. Preliminary results indicate that the catalytic domain-chitin and insert chitin domain (CAT-CID) have Km and Vmax of 3.32 uM 5.53 nmol/min, respectively; whereas the fibronectin-catalytic (CAT/FN3) domain shows Km of 5.54 mM and Vmax of 13.9 nmol/min. We have developed different construct, including the catalytic domains (CAT), chitin binding domain (CMB2), and chitin insert domain chitin (CID), and we are in process of characterization using different protocols such as chitin binding assays and zymograms.

**Keywords:** *Bacillus thuringiensis*, ChiA74, three-dimensional structure, domains, chitin.

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# The methylotrophic yeast *Pichia pastoris* is a suitable host to express complex antigens from *Mycobacterium tuberculosis*

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Pulmonary tuberculosis is a global health concern caused by the pathogen *Mycobacterium tuberculosis*. It affects about one third of global population; however, progression to the active phase of the disease is influenced by factors such as diabetes, malnutrition, HIV, alcoholism, among others. In 2015, 9.6 million new infections were reported by the WHO, and 1.5 million deceased due to complications. Actual vaccine BCG is effective against the initial pulmonary infection between 0 to 80% of the cases, while diagnostic procedures often results in unspecific reactivity caused by non-infective mycobacteria in the environment. *M. tuberculosis* produces a variety of antigens isolated from the culture supernatant of the bacillus; some of them are glycoproteins that are proposed in the development of a multivalent vaccine or in diagnostic kits. Mannosylated antigens are special because depending of their pattern, it acquires different immunological properties. However, the growth biohazard of *M. tuberculosis* and the purification of these antigens make the process unsuitable for further studies, characterization and for biotechnology bioprocess development. To overcome the drawbacks of this bio-safety level 3 organism, the three antigens were expressed in the methylotrophic yeast *Pichia pastoris*. Three plasmids were constructed with the optimized sequence codifying for each antigen under the AOX promoter, which is induced by the presence of methanol in the culture media. The antigens were identified by SDS-PAGE and Western Blotting from the culture supernatant. Using a lectin blot with ConA/HRP assay; we demonstrated that the antigens are mannosylated. The purification assays of the antigens were performed by rpHPLC or Anion exchange chromatography. Then, the proteins were identified by mass spectrometry fingerprint. In shake flasks, the yield of all antigens was around 100-200 mg/L. The processes in bioreactors of 1L under controlled conditions are currently in progress with the aim to characterize protein production yield, antigen structure-function and its immunological properties. In this work, we remark the utilization of the yeast *P. pastoris* as factories for the production of mannosylated antigens from *M. tuberculosis* that could potentially be used for biomedical purposes.

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# Expression of *Vitreoscilla stercoriaria* hemoglobin improves growth and reduces lactate yields in CHO-K1 cells

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***Background and novelty.*** Chinese hamster ovary cells (CHO) have remained over time as the most widely used expression system for biopharmaceuticals production. However, a high lactate excretion rate during the culture of these cells is still a problem. Accumulation of this metabolite in media produces adverse effects in cell growth, productivity and viability. Strategies to control lactate production include media and feed batch process optimization and cell engineering. In this way, a different strategy is proposed in this work, expressing the *Vitreoscilla stercoraria* hemoglobin (VHb) in CHO-K1 cell line. VHb expression has been related with cell growth and protein production improvement, and also has a positive interaction with energy metabolism. For this reason, we expect that VHb could help to reduce lactate excretion in CHO cell cultures. There are several reports about VHb expression in different organisms including bacteria, yeasts and plants but, there is only one report in CHO cells and it was focused on post-translational processing acceleration.

***Experimental approach.*** As a first approach, we used transient expression with Lipofectamine 2000 to evaluate the VHb effect on CHO-K1 cells. VHb gene (*vgb*) was modified for expression in CHO cells and finally cloned into pVAX-1. Besides, plasmid pVAX-1 with the green fluorescent protein gene (GFP) was used as a reporter plasmid for transfection efficiency determination. VHb expression was verified by western blot analysis. Additionally, fluorescence microscopy was performed for intracellular localization of the VHb. Finally, cell growth curves after transfection with pVAX-*vgb* and pVAX-GFP were carried out, and glucose, glutamine and lactate were measured. Cultures with and without Lipofectamine were used as controls.

***Results and discussion.*** According to western blot results, VHb was successfully expressed in CHO-K1 cells after transfection with pVAX-*vgb*. Also VHb was found throughout the cytoplasm and in intracellular structures, but further analyses are needed to determine if VHb could be located also in mitochondria, where it is expected to deliver oxygen. Cells expressing VHb exhibited a 36% greater specific growth rate than cells expressing GFP, which is coincident with reports about VHb improvements in cell growth. Also glucose and glutamine were more efficient utilized by cells expressing VHb than by cells expressing GFP since the values for yields of biomass on glucose and glutamine ($Y_{x/s}$ and $Y_{x/gln}$) were higher in cells transfected with pVAX-*vgb*. Besides lactate produced per cell was lower in cells expressing VHb than in those expressing GFP. However, no difference was found in specific glucose and glutamine uptake rates ($q_s$ and $q_{gln}$) and specific lactate excretion rate ($q_{lac}$) between those two groups of cells. This is an effect of the increased growth rate when VHb was expressed. Such results are relevant for practical applications and can potentially be improved by increasing transfection efficiency or constitutive expression of the *vgb* gene. Altogether, our results show that aerobic expression of the VHb is a useful tool to improve the growth performance of CHO cells.

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# Analysis of the role of the peroxidase ZmPrx35: an insight to the biochemical mechanisms of post-harvest insect resistance in maize *(Zea mays L.)*

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Maize (Zea mays L). is one of the most important crops for human nutrition and animal feedstock. Mexico has been considered as the origin and diversification centre 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. With the emerging use of maize as raw material for 'bio'-fuel generation, its demand has increased in the last years, causing rising of the cereal price, and affecting net-importers of this basic food, such as Mexico.

Forty percent of the worldwide maize production is lost due to the action of biotic and abiotic factors. One of the main causes of post-harvest losses in tropical and sub-tropical areas is the attack by insects, mainly of *Prostephanus truncatus*, *Sitophilus zeamais* and *Sitotroga cerealella*. Reduction of these losses could significantly improve the availability of maize in disadvantaged regions.

In recent years, it was found that phenolic acids and peroxidases contribute significantly to the resistance of maize kernels against insect pests. However, the role of these compounds in the mechanisms that confer this resistance 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 is still unknown. Therefore, in this work we aim to determinate how this POD could be implicated in the kernel resistance to the insect attack, using a combination of histological methods, activity-directed proteomics, and molecular biology tools.

Our findings will contribute to the screening for insect resistant maize varieties and will support marker-assisted breeding.

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# Immobilized laccase-mediated free radical polymerization of acrylamide in deep eutectic solvents

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The concept of green chemistry has become relevant in organic, inorganic and polymer synthesis due to the fact that many chemical reactions involve the use of toxic and non-biodegradable compounds as precursors, reaction media and catalysts (Sánchez-Lejía *et al*. 2016). In chemical and biochemical reactions, the selection of the correct solvent is essential, since it constitutes around 80% of the total volume of the reaction, but most of the organic solvents commonly used do not fulfill the requirements to be considered green technology, since they have an inherent toxicity and high volatility (Fischer 2015). In this order of ideas, many aspects have been considered to address the problem. The use of enzymes as catalysts to replace the inorganic catalysts which have the advantage of their high specificity and recycling properties through immobilization (Anastas *et al*. 2000). Among the fungal enzymes, the laccases offer more stability, a extensive amount of substrates, the possibility to utilize the enzyme in its inmobilized form, and their reactivity can be expanded by means of mediators such as 2,4-pentanodione; this mediators modify reactivity towards other substrates wich laccase alone cannot oxidized. The change of solvents used as reaction medias that fulfill the 12 principles of the green chemistry (Anastas y Kirchhoff 2002). Some of those studied medias are water, ionic liquids (Clark y Tavener 2007) and deep eutectic solvents (DES) (Paive *et al*. 2014). DES can be obtained from the mixture of a quaternary ammonium salt with a hydrogen bond donor, such as an amine, carboxylic acid and polyalcohols(Dai *et al*. 2013). These reaction medias exhibe lower freezing points than their precursors, they are biodegradables, cheap and easy to synthetize. Another DES characteristics are their potential use as catalyst, selective polymer dissolution capacity, which make them useful in the synthesis of polymers and the stabilization of some enzymes (Durand *et al*. 2013). One of them is the choline acetate:glycerol DES, that have been proved as reaction media with peroxidase as catalysts to free radical polymerization (Sánchez-Lejía *et al*., 2016). Motivated by these findings, we studied the effect of the choline acetate:glycerol (ChAc:G) DES in a 1:1.5 molar ratio, over the catalytic activity of *Trametes versicolor* immobilized laccase to polyacrylamide free radical polymerization. The reaction conducted in DES-aqueous mixtures at different concentrations (% v/v) of acetate buffer 0.1 M pH 5, using 2,4-pentanodione as initiator and enzyme substrate. We found that *Trametes versicolor* laccase can be immobilized in silica gel, without the total lost of his activity, just like other authors have reported. The enzimatic activity of free and immobilized *Trametes versicolor* laccase was tested in the DES-aqueous mixtures.The highest enzyme activity was obtained when the buffer solution was increased.

# Stabilizing effect of the V915L mutation on the reductase domain of engineered cytochrome P450 BM-3

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Cytochrome P450 enzymes are heme-containing proteins known for their efficiency to hydroxylate inactivated alkanes. Eukaryotic members of the P450 superfamily are typically associated to membranes, which makes them insoluble and difficult to characterize. The di-flavo-cytochrome P450 (BM-3) is a protein of Bacillus megaterium in which the cytochrome P450 fatty acid hydroxylase and the di-flavin reductase NADPH-dependent merge into a single polypeptide. Unlike the mammalian P450 redox systems, the P450 BM-3 is soluble and the fused catalytic domains allow it to have the highest catalytic activity among all P450 monooxygenases (turnover number for arachidonic acid of 17,000 per minute). Cultures of E. coli cells expressing the BM-3 have been used to produce specific hydroxylated fatty acids, thus demonstrating the potential of the enzyme for the production of a range of chiral pure compounds. Therefore, the catalytically self-sufficient cytochrome BM-3 is of great interest from an industrial point of view, limited only by the low stability of its reductase domain. Specifically, the FMN-binding domain exhibits thermal instability, with a Tm app value of 45 °C.

We constructed a multiple mutant, based on a consensus mutagenesis approach, that showed an important increase in stability. The enzyme contains FAD, FMN, and heme cofactors. Each of them located in separated domains, thus the circular dichroism (CD) signals at specific wavelengths (in the range of 220 to 475 nm), can be used to monitor the structure of each of the cofactor-binding domains, the secondary structure content and tertiary interactions. The apparent thermal stability of the mutant was monitored in the temperature interval of 15 to 80 °C, showing a 10 °C increment in the unfolding transition of the FMN-binding domain, compared to the WT enzyme. While variants containing other mutations at the reductase domain exhibit two unfolding transitions, one for each of the FMN and FAD-binding domains; the multiple mutant increased the unfolding cooperativity of the reductase domain, to give a single conformational transition. To the best of our knowledge, this is the first mutation that significantly increases the stability of the FMN-binding domain of BM-3.

# Enzymatic profiling of oxidases produced by *Oxyporus latermaginatus* in submerged fermentation in the presence of lignocellulosic extracts

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During the past decade he developed a keen interest in the use of white rot fungi (WRF) for purposes of bioremediation, this due to its ligninolytic enzyme systems, such as lignin system degradation of these fungi does not have specific substrates, are capable of transforming and sometimes completely mineralize a variety of environmental contaminants. The production of these enzymes is favored in submerged fermentation and incorporation of lignocellulosic extracts. In this paper was evaluated the effect of extracts of wheat straw (EWS) on activity enzyme in submerged fermentation of *O. latemarginatus*, using different concentrations of ethanol (50, 70 and 96%) as solvent and water by strirring. The maximum of biomass was obtained in the fermentation with 50% ethanol (7.51 g/L), while the minimum of biomass was presented in basal fermentation (3.43 g/L). On the other hand, the fermentation with 70% ethanol had the highest growth rate (0.079 $h^{-1}$) while the least growth rate was obtained in the fermentation with ethanol 50% (0.03 $h^{-1}$). The enzymatic activities were quantitated by spectrophotometry UV, the LiP and VP highest activity was obtained in fermentation with 50% ethanol of 2424.72 IU/L to 216 h and 534.05 IU/L to 432 h respectively, on the other hand from the enzyme MnP the highest activity was obtained in the fermentation with water extract (761.58 IU/L) to 216 h with respect to enzyme DyP activity 604.66 Ul/L present to 336 h treatment with 96% EtOH. Finally, for the case of Lac enzyme was not a significant effect on the enzymatic activity of this enzyme by any treatment. Inductive effect was observed with the extract 50 y 75%. This research was supported by grant SIP 20161426 from Instituto Politécnico Nacional.

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# Study of scale-up criterion kLa and Pg/V in bioprocess for recombinant expression of dextranase in *Pichia pastoris.*

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**Introduction:** in the process of scale-up of aerobics bioprocess, the oxygen transfer ratio is the main parameter to keep during the scale-up from bench to production scale (Junker, 2004). After keep geometric similarity in the bioprocess system through scale-up, the oxygen transfer rate (OTR) must keep constant; due this it's proposed use scale-up criterion which describe the oxygen transfer phenomenon, like volumetric mass transfer coefficient (kLa) and the power supply under aerated conditions (Pg/V) (García-Ochoa & Gomez, 2009); *Pichia pastoris* is a methylotrophic yeast strictly aerobic with huge relevance in the recombinant protein expression, however there are little previous literature in which has been characterized the behavior of the yeast trough scale-up process further bench scale.

**Objective:** analyze the effect of different values of kLa and Pg/V on the recombinant dextranase expression in *P. pastoris* at bench scale, the results will be use for the scale-up of the bioprocess.

**Experimental:** we used a *P. pastoris* Mut$^{s}$ strain that express a recombinant dextranase, under different operation conditions for the change of values of kLa and Pg/V in submerged fermentations using a stirred tank bioreactor filled with FM22 defined medium under a fed-batch process. During de time of fermentation until the end; dextranase activity, biomass and total proteins were measured in clarified broth.

**Results:** with kLa values of 138, 150 and 185 $h^{-1}$ (and a constant coefficient Pg/V of 0.4 W/L), the fermentations developed under oxygen limited conditions (OLC); this strategy has been used und characterized like an option for robust bioprocesses with adequate expression yields (Gurramkonda et al., 2009). The results obtained show that dextranase activity in the clarified broth is the same in the three values of kLa; however, it seems that an increase in the kLa has a positive effect in the biomass and protein production. Our enzymatic activity is lower than other previous reports; this has been attributed to liberation of proteases from the cultivated yeast.

**Conclusions:** changes in kLa values for submerged fermentations have a positive effect in the production of biomass and proteins, inclusive under oxygen limited conditions. It's possible the limited aerated conditions observed, have a negative effect in the expression of the recombinant dextranase due to stress effects on the yeast.

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# Nanovesicles from eukaryotic cells produced by acoustic cavitation

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Extracellular vesicles (EV) are nanovesicles secreted from cells, carrying genetic and proteomic information to recipient cells. Their therapeutic potential is large and has boomed in recent years, including their proposal as vaccine and drug targeting vehicles.

Purification of EV is usually inefficient and involves cumbersome steps, which has importantly limited their research for pharmaceutical applications. In this work, shock waves-induced acoustic cavitation, one of the principal mechanisms in extracorporeal lithotripsy, was explored as a tool for producing EV from HEK293 and NIH3T3 immortal cell culture lines, as well as from primary cultures of mouse T cells.

Tracking and ultrastructural characterization of nanometric vesicles was performed by dynamic light scattering and transmission electron microscopy. The stability and bioactivity of molecules to be either encapsulated or displayed by EV were assessed by various tests, including agarose gel-electrophoresis and CD antibody labeling. Our results show that shock waves induced the production of vesicles 30 to 40 nm in diameter, at physical parameters proving to be compatible to nucleic acid stability. More tests are being performed to explore the potential of these vesicles for therapeutic applications.

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# PRODUCTION AND PURIFICATION OF THE PROTEINS NEP Y NS1 OF INFLUENZA AH1N1 VIRUS IN *E. coli*

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Influenza is an acute respiratory and highly contagious illness. In 2009 was detected an emerging strain of influenza virus H1N1 in Mexico, which has spread to more of 214 countries. The influenza A virus has a segmented negative RNA genome, the 8° segment encodes to Non Structural Protein (NS1) and by splicing the Nuclear Export Protein (NEP). In our laboratory Lara-Sampablo reported that in transfected cells with NEPpdm, the TNF-$\alpha$ promotor activity were increased, the TNF-$\alpha$ protein (highly expressed during influenza virus infections and is considered an anti-influenza cytokine) biosynthesis was induced. In the same report, it was found that when the TNF-$\alpha$ promoter distal region was deleted, the promotor activity decrease suggesting a role of the Raf/MEK/ERK in the regulation of the transcription. However, the mechanism whereby NEP active TNF-α is unknowing. The cellular localization of NEP and NS1 must be determinate.

To continue with the study of properties and interaction at cellular level of NS1 and NEP, in this project were constructed recombinant influenza virus proteins NS1 and NEP in E.COLI BL21 with the plasmids pQ-NEPpdm09 y pQ-NS1pdm09, that have a selection ampicillin gen, LAC promoter and a Histidine motif (6X). Strains BL21-NS1 Y BL21-NEP were growing in LB medium. The expression was induced by IPTG. The highest presence of recombinant proteins (NEP, NS1) in inclusion body was confirmed by PAGE-SDS y Western-blot with antibody anti-His, the inclusion body were solubilized with urea 8M. The recombinant proteins were purified by affinity chromatography Ni-NTA column (IMAC) observing with a PAGE-SDS and Western-blot that the protein NS1 (26 kDa) and NEP (15 kDa) were eluted with highly efficient and high degree of purification. Proteins retain their properties to block the pathway of signal transduction of IFN as well as NEP the ability to decrease the activity of the promoter which encodes to TNF-$\alpha$. Proteins retain their properties in the viral infection process as well as NEP the ability to decrease the activity of the TNF-$\alpha$ promoter.

- Alejandra Lara-Sampablo, Nereyda de Jesús-Ortega, Gerardo Santos-López, Verónica Vallejo-Ruiz, Nora Rosas-Murrieta, Sandra Reyes-Carmona, Irma Herrera-Camacho, Julio Reyes-Leyva. Transfection of Influenza A Virus Nuclear Export Protein induces the expression of Tumor Necrosis Factor alpha. Virus Research. 185: 1-9. 2014. DOI: 10.1016/j.virusres.2014.03.011.

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# Cloning and Expression of mutant defensins with specific binding to phosphatidic acid.

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Plants are sessile organisms that are exposed to numerous stress factors either abiotic or biotic. As innate immune response, defensins are the class of peptides that appears to be conserved among plants, invertebrates and vertebrates. Plant defensins are acidic peptides with antifungal activity formed by 45 to 55 amino acid residues with eight cysteines that form four disulfide bridges. Plant defensins belong to the alpha beta cysteine stabilized (αβCS) structural family, as other cysteine rich peptides. The region in the C-terminus defined as the γ-core, is the motif known to interact with phospholipids for peptide internalization through the plasma membrane and is also involved in antifungal activity (De Samblanx *et al*., 1997). CADEF1, a defensin isolated from a cDNA library of *Capsicum annuum* show 83% of identity with MtDEF4, which is a defensin of *Medicago truncatula* that binds phosphatidic acid. CADEF1 and MtDEF4 share an identical sequence, CRGFRRRC, in theγ-core. Therefore two mutants in the γ-core of CADEF1 (CADEF1_AARR and CADEF1_AAAA) heterologously expressed in bacteria were designed to determine if both mutant defensins bind to phosphatidic acid or not and if they show antifungal activity.

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# Study and bioinformatic characterization of *omp6, omp7, omp8, omp9, omp10* genes from Mexican strains of *Anaplasma marginale*

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Keywords: *Anaplasma marginale*, Outer membrane proteins, genetic diversity.

*Anaplasma marginale* the causal agent of bovine anaplasmosis is an obligate intracellular, gram-negative bacteria, from the order of Rickettsiales. *A. marginale* infects mature erythrocytes with the formation of a vacuole derived from these erythrocytes. The outer membrane proteins of *Anaplasma* are not only essential for the bacteria but also serve as main objectives of the innate and adaptive immune response and therefore may be relevant to vaccine development. The nucleotide and amino acid sequences of *omp6 - omp10* genes from St. Maries strain and from eight Mexican strains of *A. marginale* previously characterized with regards to MSP4 and MSP1a were used to bioinformatic analysis first for basic physicochemical characteristics such as molecular weight, isoelectric point. The sequences were compared among themselves and analyzed for functional domains, possible location within the cell, molecular structure, relationship with nearby genes, phylogeny, presence of signal peptide, secondary structure prediction, hydrophobic profile, transmembrane domains, globular region analysis, and presence of type B epitopes and antigenic regions. Our results show that there is little diversity between *omp* genes from the different strains. There were predicted linear type B epitopes located on the extracellular domains that are highly conserved. These omp's are classified as porins belonging to superfamily of OM_Channels. All but OMP10 include signal peptide, and the putative mature proteins are located on the outer cell membrane. Sequence alignment of both nucleotide and amino acid sequences showed that these genes and their putative proteins are highly conserved. While there were deletions and substitutions, the great majority of important epitopes were retained. Therefore, the amino acid sequences of *omp* genes meet the desirable characteristics of a vaccine protein.

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# Isolation of hydrocarbon degrading bacteria from diesel and gasoline contaminated soil at La Comarca Lagunera

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## Abstract

Bioremediation has become the most important mechanism for removing oil and its derivatives because of the widespread problem caused by discharge and accidental spillage in soil as well as in water environments. The main aim of this study was to isolate bacterial strains with the ability to degrade hydrocarbons from diesel and gasoline. Strains were isolated in Luria Bertani broth enriched with 1% of hydrocarbons (diesel and two types of gasolines) which were subsequently grown in Bushnel Hass media with a sole carbon source. Several conditions were proved to find optimal growth rate, kinetic studies for growing and production of extracellular protein were daily measured during fifteen days. Isolates and their treatments (D1-A27, D2-B19 & D3-C35), were tentatively identified as members of the genera *Bacillus* and *Streptococcus* species based on their biochemical and morphological characterization which shown that the strain D2-B19 (pH 5 and 0.5% Diesel as carbon source) had the best growth rate (0.247 OD). Also, related with its protein production (120 mg/L) at the tenth day of incubation, followed by D1-A27 (pH 5 and 1.5% RG as carbon source) & C35 (pH 7 and 1.5% GG as carbon source) respectively. The optimal condition for supporting growth was pH 5. From this study, we can say that the isolates have the ability to use hydrocarbons from fuels as a sole carbon source.

**Key words**: *Kinetics studies, hydrocarbons, growth rate and strains*

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# *Solanum lycopersicum* interaction with a *Trichoderma atroviride* strain that overexpresses an expansin type protein.

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Fungi of the genus *Trichoderma* spp. are known for their promoter growth activity on plants and producing organic acids that can decrease the pH of the soil and allow solubilization of phosphates and micro and macro nutrients such as iron, manganese and magnesium which are vital for plant metabolism(1). Penetration root tissue is generally confined to the first or second cell layers and only in the spaces. Expansin and expansin-like proteins such as swollenin, are proposed to loosen the cell wall due to the breaking of hydrogen bonds among the filaments of cellulose or between cellulose and other polysaccharides (xyloglucans) by a non-enzymatic mechanism (2). *Trichoderma* also can activate the defense mechanisms of plants, thus conferring resistance to biotic and abiotic stress (as can be temperature conditions).
In the laboratory Atriztan et al, built a *T. atroviride* strain which overexpresses its own swollenin. This strain was evaluated for tomato plants colonization, obtaining higher biomass production (leaves, height, root) compared to strain WT *T. atroviride* and non-inoculated plants. When plants were subjected to cold and heat stress, favorable results were obtaind for the overexpressor strain (Ta swoi-28) as it showed greater survival.

Phenotype obtained by non-inoculated plants, WT,*T.a Swol-28* and survival to heat stress.

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# Study of the interaction and permeability originated by PLGA nanoparticles loaded with melittin in phospholipid membranes that resembles mammary epithelial and breast cancer cells

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In spite of great advances in cancer therapy, there is considerable current interest in developing anticancer agents with a new mode of action because of the development of resistance by cancer cells towards current anticancer drugs. A growing number of studies have shown that some of the cationic antimicrobial peptides (AMPs), which are toxic to bacteria but not to normal mammalian cells, exhibits a broad spectrum of cytotoxic activity against cancer cells. Melittin is a water-soluble cationic amphipathic 26-aa alpha-helical peptide derived from the venom of the honeybee *Apis mellifera.* The $NH_2$-terminal region of melittin is largely hydrophobic whereas the region at the COOH-terminus contains positively-charged amino acid residues and is hydrophilic. Melittin forms channels in lipid bilayers and is lytic for both cancer cells and normal, healthy cells including erythrocytes. Because of the relative lack of selectivity displayed by melittin for cancer cells, its therapeutical potential cannot be achieved without a proper delivery vehicle, which could be overcome by melittin nanoparticles that possess the ability to safely deliver significant amount of this cationic peptide intravenously and to target and kill tumors. In this work we evaluated the membrane disruptive effect of PLGA nanoparticles loaded with melittin on lipid vesicles that resembles mammary epithelial and breast cancer cells using the ANTS/DPX assay. We show that membrane permeabilization is mainly determined by the presence of negatively charged lipids suggesting that electrostatic interactions play an important role. The results will be discussed in terms of the anticancer potential activity of this lytic peptide.

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# Study of the overexpression effect of the Rsm sRNAs and its effects in alginate production in *Azotobacter vinelandii*

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## Introduction

*Azotobacter vinelandii* is a soil, nitrogen fixing bacterium, pleomorphic having up to 80 copies of its own chromosome, belonging to the *Pseudomonadaceae* family, which it can enter in differentiation stage forming desiccation cyst resistant.[5] This bacterium produce lineal polymers constitute for mannuronic acid and its epimer guluronic acid, callings alginates which are from biotechnological interest.[1] Its regulation synthesis is focus in the GDP mannose dehydrogenase activity, encoded by *algD* gene [3]. *algD* is regulated by GacS/GacA two component system [4] altogether with Rsm, a posttranscriptional regulation system. The Rsm system is composed by a repressor protein (RsmA) and small non–coding sRNAs.[2]

In *A. vinelandii* has been reported the existence of nine sRNAs belonging to Rsm family (8 from Z subfamily and 1 from Y subfamily). The expression of all the sRNAs is GacA dependent. The participation of RsmZ1 and RsmZ2 in the alginate synthesis has been characterized; mutations of these sRNAs reduce the alginate synthesis in a 80%. However, the effects of the mutation of the other sRNAs genes affect alginate production in different extent. This suggests that all the sRNAs play a role in the alginate synthesis. Mutations in *gacA* abrogate totally the alginate synthesis. Likewise, it has been seen that the constitutive expression of *rsmZ1* (independently to GacA) is be able to restore its production.

To study, individual and functionally, each sRNA we overexpress the sRNAs in a *gacA* mutant, which no expresses any Rsm sRNA. To express constitutively the sRNAs we constructed an integrative cloning vector, this vector contain a *gyrA* constitutive promoter, a multicloning site and and a promoting recombination locus. Using this molecular tool we found that the overexpression of any Rsm sRNA restore the production of alginate in a *gacA* mutant.

In previous studies in our group, we have been shown that mutations in each one of sRNAs affect alginate production in different extent, also we discover that all of them are expressed in the optimum conditions for alginate production, this suggest that all the sRNAs play a role in the alginate synthesis we have demonstrated newly that the *gacA* mutant production where overexpressed independent to GacA,.

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# Characterization of native chitinases from marine microorganisms and their possible application in biotechnology in biological control

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Chitin, is a polymer formed by residues of N-acetyl-D-glucosamine (GLC-NAc) linked by ß (1-4) bonds, this polymer is the second most abundant compound on earth[3]. It is located mainly in the marine environment forming part of the exoskeleton of crustaceans, other invertebrates and algae[1]. There is a large number of organisms which have developed the ability to produce enzymes which degrade the chitin; they are called chitinases[4]. They are produced by different microorganisms, in particular, by marine bacteria, and it has been shown that they can be the very efficient in the degradation of chitin. The great importance of chitinases in the degradation of chitin has generated a diversity of biotechnological applications and uses mainly in the field of agriculture with the production of bio insecticides; production of algaecides; as well as in the pharmaceutical industry in the production of pharmaceuticals drugs[2,5].

The objective of this research project is to isolate and identify chitinase producing organisms from marine samples and measure their chitinolytic activities. Additionally, the native chitinases produced by these organisms will be biochemically characterized and applied in bioassays for controlling pests affecting crops of agricultural importance, or as algaecides, and thus, help populations affected with those problems.

Twelve colonies with chitinolytic activity were isolated and identified after analyzing a total of 50 samples of sea water from the Gulf of Mexico. DNA 16S sequencing revealed that the strains corresponded to the genera of *Pseudoalteromonas*, *Vibrio* and *Photobcterium.* Halos of chitin degradation with different diameters were observed when the isolates were grown in a minimal marine medium, complimented with chitin. This suggests the presence of a variety of chitinolytic activities in these bacteria and that there is potential for application in biotechnology in to help control the problems mentioned above.

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# Prolactin and 17β-Estradiol Induce Pro-Inflammatory Cytokines in Bovine Mammary Epithelial Cells Inhibiting *Staphylococcus aureus* Internalization

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Cattle are more susceptible to infectious diseases during the peripartum such as mastitis. This period is characterized by drastic changes in the concentrations of 17β-estradiol (E2) and prolactin (PRL), which compromise the innate immune response (IIR) of the animal. Bovine mammary epithelial cells (bMECs) are target tissue of these hormones, which regulate their proliferation and differentiation. In addition, previous work from our group shows that these hormones have immunomodulatory effects on bMECs: PRL induces (5 ng/ml) the internalization of *Staphylococcus aureus* (principal mastitis pathogen) and E2 decreases it (50 pg/ml). The IIR (cytokines, chemokines, antimicrobial peptides, etc.) stimulated by PRL in bMECs is inhibited in the presence of *S. aureus*, and E2 induces elements of the anti-inflammatory response in bMECs. *S. aureus* is able to internalize using the "zipper" mechanism associated to α5β1 integrin in the host cell. In spite of both hormones play a relevant role for bovine mammary tissue *in vivo* their combined effects on the IIR of bMECs during infection are unknown. For these reasons, we hypothesize that the hormonal combination (mixture) down regulates the IIR of bMECs during *S. aureus* internalization. In the present work, we also explore the inflammatory signal transduction pathways that can be regulated by the mixture in bMECs. For this study, we used 17β-estradiol (Sigma) and bovine prolactin (A.F. Parlow-hormone program from NIH) in primary cultures of bMECs isolated from a lactating cow, and the *S. aureus* strain ATCC-27543 isolated from a clinical case of mastitis. bMECs were treated 24 h with the mixture and then were infected 2 h with *S. aureus*. Viability assays for bMECs treated with mixture were determined by MTT reduction and trypan blue exclusion assay; bacterial viability was analyzed by CFU counting and flow cytometry (FC). The invasion was analyzed using gentamicin protection assays; FC was employed for receptor and kinase analysis, and RT-qPCR and ELISA assays were used to determine the expression of IIR genes and protein secretion, respectively. The results indicated that the mixture did not modify bMECs or bacteria viability after 24 h. The mixture decreased *S. aureus* internalization (~50%) in bMECs. This effect was associated with a membrane reduction of integrin α5β1 abundance (~80%). The mixture inhibited p38 and ERK kinases activation compared with control bMECs. In addition, the secretion of the antimicrobial peptide DEFB1 increased in the presence of the mixture and *S. aureus* (~2 fold), with respect to infected bMECs. The gene expression of the pro-inflammatory cytokines IL-6 and TNF-α were up-regulated in the presence of the mixture (~4 and 12 fold, respectively). In conclusion, the hormonal combination of PRL and E2 induces elements of the pro-inflammatory response in bMECs, while reducing *S. aureus* internalization. It is necessary to elucidate the signal pathways involved in these effects since MAPK activation does not participate.

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# Elicitors for the production of steviosides from cells suspension of *Stevia rebaudiana* Bertoni.

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*Stevia rebaudiana* Bertoni. is an important plant for producing sweeteners (steviosides), which do not provides calories. The growing demand for these compounds leads to seek alternative techniques of productions such as cell culture in suspension and the use of elicitors. These techniques reduce time, space, labor, agricultural machinery and inputs required to produce them in traditional systems (whole plant). The objective of this work was to determine the effect of methyljasmonate, salicylic acid, light and temperature (elicitors), in steviosides production from cell cultures suspension of *S. rebaudiana*. For induction of friable callus, leaf segments and nodes were cultured in media containing basal salts of Murashige and Skoog (MS) (1962), sucrose, antioxidants, auxins (2,4-dichlorophenoxyacetic acid (2,4-D), naphthalene acetic acid (NAA) and fluorophenoxyacetic-4 acid), and cytokinins (6-benzylaminopurine (BAP)). To test the effect of elicitors, the cells were suspended in a medium with MS salts and the combination of 2,4-D and BAP at the same concentration (1.5 mg $L^{-1}$), to which it was added methyl jasmonate ( 10 to 100 mM) or salicylic acid (100 mM). Likewise, suspension cultures were grown in the presence of red, blue and white light and 25 or 28 °C. The culture medium containing the same concentration of both BAP and 2,4-D was the treatment promoting the best results in terms of efficiency to induce callus and their characteristics. The methyl jasmonate at 10 mM with white light increased considerably the production of steviosides however, culturing the cells in suspension at 28 °C or salicylic acid 100 mM increased up to nine fold the concentration of these glycosides. It is concluded that by manipulating the conditions in which the cells of *Stevia rebaudina* are grown may increase the stevioside production.

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# PepGMV tolerance induction in pepper plants with hydrogen peroxide solutions

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Foliar applications of hydrogen peroxide ($H_2O_2$) in young plants of *Capsicum annuum* L. were performed, in order to evaluate the role of this elicitor in induced systemic resistance (ISR) leading to tolerance and / or resistance to stress biotic in plants. $H_2O_2$ concentrations used were 6 mM, 14 mM and 18 mM and, CAT and PAL enzyme activities were evaluated. as well as expression of transcripts cat1, pal, as part of oxidative stress generated by the elicitor. Likewise, transcripts of mkk1, mk1, mk2, wrkyd, wrky1, npr1, pr1 involved in intracellular signaling pathway MAP kinases were evaluated by RT-PCR as part of the interaction *C. annuum* L-Elicitor-Biotic stress (PepGMV). Our results show that the foliar application of $H_2O_2$ elicitor in *C. annuum* L., induces ISR; which could be verified with the high endogenous content of $H_2O_2$ (5 ng / mg wet weight) generated by foliar application of 18 mM $H_2O_2$, compared to water control and tolerant phenotype to PepGMV with the same elicitor treatment. Similarly, it was confirmed that the elicitor foliar application, induces the expression of genes involved in the main intracellular signaling pathway, MAP kinase.

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# Evaluation of the modulatory effect of peptides with affinity to human protein disulfide isomerase (PDIA1)

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During protein biosynthesis, folding is an essential process to get the functionally active structure. In eukaryotic cells, secretory proteins attain their native conformation mainly in the endoplasmic reticulum (ER), where a vast assortment of molecular chaperones and foldases assists the process. In human cells, among the most abundant ER-residents is the foldase PDIA1 (protein disulfide isomerase A1), which is an oxidoreductase that catalyzes the formation and rearrangement of disulfide bonds in nascent polypeptides. Also, it exhibits chaperone-like activity, being important to prevent aggregation of misfolded intermediates. Intriguingly, numerous human pathologies, including some proliferative, neurodegenerative, metabolic, and vascular diseases, have shown a differential profile of PDIA1 expression, favoring disease progression. So far, commercially available inhibitors are not appropriate for clinical use, due to their low specificity and high toxicity. Moreover, there have been no reports on molecules with activator effect. Hence, it is imperative to develop experimental approaches aimed to identify new molecules with a specific effect on PDIA1 (inhibitor or activator) and therapeutic potential. Through a phage display approach, a peptide library was screened for binding to human PDIA1. Three heptapeptides with affinity to this molecular target were identified. The plausibility of each peptide as an effective modulator is now being examined by *in vitro* assays. To date, results on modulating the reductase activity have shown promising outcomes. Based on their binding parameters ($EC_{50}$, $K_d$, $IC_{50}$, $K_i$), the sequence of the best-scored peptide will be used as the structural core for a rational drug design approach, aimed to develop new modulators of PDIA1 with high specificity and low toxicity, and intended to control human diseases.

# Metabolic Signatures of Tomato Plants with Differences in Jasmonate Biosynthesis

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Plants have developed multiple strategies to defend themselves against herbivory and microbial infections. Jasmonic acid (JA) is a well-known hormone involved in plant defense. It is synthesized from linolenic acid and its accumulation is induced by mechanical plant damage.

In this study, we analyzed the influence of JA biosynthesis on secondary metabolism. We studied the tomato cultivar Castlemart and two genetically modified derivatives: a suppressor of the prosystemin-mediated response mutant (*spr2*) that is compromised in linolenic acid biosynthesis and JA synthesis, as well as a prosystemin over-expressing plant (*35S::PS*), which is characterized by an over-regulated defense response[1, 2].

We performed metabolic characterization by direct measurements from the plant surfaces using a Low Temperature Plasma (LTP) ionization source. This novel sampling method for mass spectrometry was developed in our laboratory and allows us to acquire the metabolic fingerprints of the volatile fraction under ambient conditions[3].

Our results demonstrate that the different genotypes also exhibit distinct metabolic signatures in their volatile composition. The mutant *spr2* is the most distinct, compared to its genetic cv. Castlemart background and the prosystemin overexpressing plant. Most detected molecules are low molecular weight compounds (< 300 *m*/*z*). But it was also possible to detect an unreported metabolite, which accumulates rapidly in response to mechanical damage.

Summarizing, direct analysis of plants by low-temperature plasma ionization MS represents an efficient tool to differentiate transgenic plants without prior sample treatment, and supports the discovery of marker metabolites.

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# Continuous monitoring of volatile organic compounds (VOCs) emitted by tomato plants (*Solanum lycopersicum)* during their interaction with pathogens

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Plants are sessile organisms and therefore are continuously attacked by a wide range of other organisms which sometimes cause diseases. The plants evolved defense mechanisms to respond to potential threats. It is well known that Volatile Organic Compounds (VOCs) emitted by plants act as signaling molecules for communication and protection1. Thus, they can be used as markers to detect infected plants in greenhouses2.

The conventional analysis of VOCs from plants is performed by Gas Chromatography – Mass Spectrometry (GC-MS). However this technique requires to sample the headspace fraction, to then separate the compounds and to finally analyze them with a mass spectrometer. This analysis is time consuming and the exact time behavior of VOC emission is difficult to model. To enable the real-time monitoring of VOCs, we assembled a Residual Gas Analyser (RGA) in our laboratory.

Our RGA is a small mass spectrometer, used to detect low molecular weight compounds in the gas phase, We were inspired by a similar system that has been used under the sea to measure VOCs released by microorganisms3.
The VOC-RGA can be used greenhouses to monitor the health status of plants. Currently, we test the efficiency of our system in the monitoring of VOCs emitted by tomato plants during the interaction with commercially relevant pathogens.

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# Determination of lignocellulolytic enzyme activity of the moderate halophile fungus *Aspergillus caesiellus* growing in wheat straw and agave fibers in halophilic conditions.

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Lignocellulosic plant biomass represents the major renewable carbon source on earth. It is considered as an environmental friendly raw material for the biorefinery industry and for other biotechnological purposes. Lignocellulosic biomass, such as agricultural residues, consists mainly of three different types of biopolymers i.e. cellulose, hemicellulose and lignin. Cellulose and hemicellulose are polysaccharides that when they converted to monosaccharides are versatile starting materials for further conversion by fermentation and biocatalysis processes to value-added products, included the enzymes that deconstruct them. Fungi, which degrade complex and recalcitrant plant polymers, secrete different enzymes that hydrolyze lignocellulose. The studies on the biomass deconstruction have been used mainly mesophile microorganisms. Recently, there has been an increasing interest in the search of extremoenzymes produced by microorganisms isolated from extreme habitats. Recent studies have shown that extremoenzymes, produced by halophilic microorganisms, exhibit some unique structural and biochemical characteristics. However halophilic hydrolases such as cellulases, xylanases, and proteases have been reported from halophilic bacteria but few have been characterized from halophilic or halotholerant fungi. Previously, we isolated a moderate halophile fungal strain that by polyphasic criteria has been identified as *Aspergillus caesiellus*. The fungus showed an optimal growth rate in media containing 1M NaCl at 28°C and could grow in media with up to 2 M NaCl. *A. caesiellus* produced cellulases, xylanases, manganese peroxidases (MnP) and esterases and differential band patterns for cellulase and xylanase activities were detected in zymograms when the fungus was grown in different lignocellulosic substrates such as wheat straw, maize stover, agave fibres, sugarcane bagasse and sawdust, however these results were obtained in the absence of salt.
Based on this background, we tested if this fungus can grow on wheat straw and agave fibers as the only carbon source with different NaCl concentrations (no salt, 0.5 M and 2 M). We determined the enzymatic activities of cellulases, xylanases, and esterases during different collection days (from 2 to 20) in order to undertand the lignocellulose deconstruction dynamic. Preliminary results show that there are higher enzymatic activities when the fungus is growing on wheat straw or agave fibers in the presence of 2M of NaCl, suggesting that *A. caesiellus* is expressing enzymes that are able to resist high salinity concentrations.

# "Visualizing calcium nano domains in living cells through optical patchclamp recording"

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Calcium plays a pivotal role as it can act as a second messenger in most cell types. It is well known that calcium signaling is tightly regulated through the generation of highly localized small transient signals. The spatial and temporal organization of calcium signals in small transients called nano or micro domains enhances the cell ability to orchestrate different simultaneous processes in tightly localized regions. Single channel activity has been widely studied using patch champ techniques, nevertheless, knowledge of these calcium elemental signals in sperm is very limited due their scarcity and cell physical constraints resulting from a complex morphology and reduced size. We are establishing the experimental methodology to record the occurrence of these signals using total internal reflexion fluorescence (TIRF) microscopy. This non-invasive methodology, which allows study single calcium channel activity in living cells, will allow us to better understand the molecular physiology of the calcium channels implicated in controlling, triggering and/or initiating physiological responses in cells with complex morphology like spermatozoa.

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# Roles of enzyme groups within each region of the intestinal tract of *E. fetida* during vermicomposting

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Hydrolases are vital enzymes for any organism because they provide metabolic capabilities to break down biopolymers and oligomers into monomers; thus the organism gets energy from available food. These enzymes perform a series of biochemical functions in every biological process, such as vermicomposting; which is a system that involves the interaction between earthworms and microorganisms. *Eisenia fetida* is an earthworm with a significant enzymatic ability to stabilize and regulate decomposition pathways of organic residues; however, the enzymatic dynamic of different hydrolytic groups of enzymes within worm's digestive tract during vermicomposting remains unknown. The aim of the study was to determine the contribution of five groups of enzymes in three different sections of the worm's digestive tract during vermicomposting. Throughout this process different enzymatic activities were observed in each gut section. In general, as a first response to fasting and 24 h post feeding, activities of most of the enzymes decreased in section C. Glycosyl-hydrolases and phosphatases showed the highest activities in the three sections of the digestive tract; while esterases, proteases, and aminopeptidases had the lowest activities. In Section A, activities of all enzymatic groups were increased at 60 and 90 days of vermicomposting, compared to 24 h-post feeding and 30 days. Section B exhibited the highest enzymatic activities of all groups, compared to sections A and C. Here, glycosyl-hydrolases and esterases might contribute to the decomposition of complex polysaccharides as hemicellulose. Section C is known as the place where nutrient absorption occurs; thus, the lowest activities of all enzymes groups were observed. These results are consistent with the chemical composition of the organic material (husks coffee with organic residues from local markets) consumed by earthworms: since there was a high proportion of fiber fractions reflected in an increment of glycosyl-hydrolases' activity; and a few content of nitrogen and fat, which resulted in low proteases, aminopeptidases, and esterases activities in the worm's intestine. This information is relevant to understand the processes occurring in *Eisenia fetida*'s digestive tract (physiology) and the enzymatic potential of this worm in the vermicomposting system for the use of organic waste.

**Key words:** Enzymes, microbiota, digestive tract, organic residues and vermicomposting.

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# Comparison of three different pH on recombinant *E. coli* BL21 (DE3) cultivation

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*Escherichia coli* is one of the expression systems most used for the production of recombinant proteins. According to the recombinant protein to be produced, a wide variety of vectors and strains can be selected. During cultivation, many metabolic changes occur, like the medium acidification, which occurs in media with glucose. This acidification is due to acetic acid formation. In any form, the factor the pH change in the culture medium has not been studied extensively. Moreover, this effect do not has been evaluated over recombinant protein production. Another problem observed during recombinant protein production is the formation of inclusion bodies. Inclusion bodies are aggregates protein consisting mainly of the recombinant protein in addition to other host proteins. The aim of this work is the study of the pH effect on *E. coli* BL21 (DE3) cultures in biorreactor under batch conditions, using three different pH values (6.5, 7.5 and 8.5).

All cultures were developed in bioreactor under controlled conditions at 37ºC, 30% of dissolved oxygen tension, and using minimal media, reached similar maximal biomass of around 8.34±1.13 g/L. Moreover, in all cultures the glucose was transformed with similar consumption rate, and when was depleted bacteria culture stops growing. The cultures at pH 6.5 presented a little increase in specific growth rate (0.97±0.02 $h^{-1}$). While cultures at pH 8.5 and 7.5 physiological specific growth rates were around 0.90 $h^{-1}$. In all cultures the recombinant protein production was observed using SDS-PAGE and western blot, and we observed all protein produced mostly in insoluble form, with same yield. We did not observed changes in bioprocesses or protein production casused by changes in pH. This information supported that the enzyme productivity will not change if the pH controllability is lost in bioreactors.

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# CRUDE EXTRACTS OF BROCCOLI (*Brassica oleracea var. Italica)* HAVE INHIBITORY EFFECT AGAINTS BACTERIA OF IMPORTANCE IN HUMAN HEALTH AND FUNGI

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Broccoli (*Brassica oleracea var. Italica*) is one of the most important agricultural products in Guanajuato, and our state is the main national exporter of this vegetable. Its importance is such that it is considered within the strategic areas of Sustainable Food innovation Industry in Guanajuato. Broccoli can be used as nutraceutical and for the development of edible membranes. In particular, antimicrobial peptides synthesized by broccoli have not been studied. Preliminary results shown that protein extracts of both broccoli floret and stem have inhibitory effect against pathogenic Gram positive and Gram negative strains of interest in food and public health, such as *Bacillus cereus 183, Micrococcus luteus, Streptococcus pyogenes, Listeria monocytogenes, Enterococcus faecalis, Staphylococcus xylosus, Salmonella spp., Escherichia coli, Shigella flexneri, Proteus vulgaris, Shigella sonnei* and *Enterobacter cloacae*. In addition, we detected the crude proteins have antifungal effect against *Aspergillus niger* and *Colletotrichum gloeosporioides.* Currently, proteins from broccoli were purified through molecular exclusion, and the inhibitory activity against *Proteus vulgaris* was analyze by gel detection, detecting that proteins of approximately 10 kDa have inhibitory effect.

Keywords : Broccoli, antimicrobial peptides , genes , edible membranes

# Biomolecular interaction analysis using magnetic particles

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MDM2 is the main regulator of the tumor suppressor p53, it acts as an E3 ubiquitin ligase, targeting p53 for proteasomal degradation under normal conditions. After DNA damage MDM2 switch to be a translation factor for p53. However, recent studies have shown that there are an increasing number of p53-independent effects ascribed to MDM2, suggesting that it has relevance in human cancer on its own. This protein is a proto-oncoprotein that plays a role in different signalling pathways since it interacts with a large variety of partners, up to now, more than 100 have been described. In this work, we use magnetic particles as a tool for detecting specific protein-protein interactions in different cellular environmental conditions. We had functionalized carboxylate-modified magnetic microspheres with the MDM2 protein produced in bacteria and, on the other hand with anti-MDM2 antibody. These particles will be internalized in H1299 cells by endocytosis, and then they will be recovered using magnetic tweezers to analyze the protein interactions. We will evaluate, by mass spectrometry and western blot the interacting proteins under normal cellular conditions and under DNA damage conditions. This technique has an advantage over other techniques, since it allows us to evaluate interactions directly in the cell unlike other methods where the molecules may interact with the whole cell lysates giving the possibility of false positives.

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# Functional analysis of a 30 kDa endochitinase by VIGS of *S. habrochaites* and *S. arcanum* species, to evaluate its possible role in defense against the bacterial canker.

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Bacterial canker in tomato is caused by *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*). This disease causes significant economic losses in Mexico and worldwide. There are wild species relative to tomato that have been reported to be tolerant to *Cmm* and represent a source of genes that could transfer to the susceptible species. Previous results obtained in our research group in wild relatives species of tomato (*Solanum arcanum* LA2172 y *S. habrochaites* LA2128) resistant to *Cmm*, showed that eight hours after infection with *Cmm*, the gene expression levels of 30 kDa endochitinase increased up to four times with respect to the susceptible commercial variety (*S. lycopersicum*); based on this preliminary result the aim of this work was the functional analysis of the endochitinase using a virus-induced gene silencing system (VIGS) based on a ToMoV geminivirus vector developed in our group, since it may play an important role in defense against the bacterial canker. The VIGS assay in *S. habrochaites* plant showed 60 percent of silencing using qRT-PCR, and it showed a more damaged phenotype compared with control plants sixty days after the infection with *Cmm* 1387 strain, these data suggest the gene silencing of 30 kDa endochitinase could be affecting the tolerance of tomato wild species due to the loss of function. Currently we are optimizing our silencing system in order to increase the silencing and reproducibility.

We also found that different *Cmm* strains may affect the tolerance pattern in different tomato wild relatives species, since *Cmm* 1387 strain showed different results to those previously reported in our group, suggesting a possible strain-specific interaction between tomato plants and *Cmm* strains that may modify the tolerance patters in wild tomato plants.

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# Comparison of sporulation of *Bacillus thuringiensis* grown by solid state or submerged fermentation

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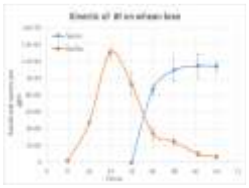
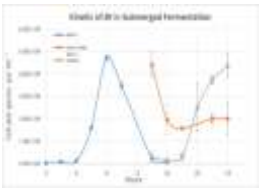
Introduction. The most successful insect pathogen used for insect control is *Bacillus thuringiensis* (Bt)(Bravo et al. 2011). In this work such comparison is made using shake flasks (SmF) and still beds of wheat brans (SSF) reporting the spore count in terms of volume or spent solids, respectively, in order to explore the scale-up calculations for either fermentation system.

Materials and methods. *Bacillus thuringiensis* var. *kurstaki* HD-73 was used. SmF. Luria Bertani (LB) medium was used for inoculum growth and a complex medium used by Rodrígez (1996) Batch cultures were performed in a Sartorius Biostat A plus reactor. SSF. Commercial wheat bran was used as substrate (5 g) with 65 % initial moisture content by mixing with .5 mL broth culture and placed in a 125 mL flask. Flasks were held at 30°C over different periods of time. Harvested material was spread on a 10 cm Petri dish and dehydrated at 60 °C for 16 h to be grounded as a fine powder. Triplicate, bacilli and spore, counts of samples were made in a Neubauer chamber

Results and discussion. Figures shows the kinetics of bacilli and spores in SmF (A) and SSF (B), respectively. In both cases bacilli curves were biphasic but peaks were at 8 h for SmF and 24 h for SSF. Spore formation started at 14 h for SmF and between 32 h and 40 h for SSF. Therefore the differentiation process was two time slower in SSF as compared to SmF. However, the maximal spore counts were much higher in SSF: $(1.15\pm0.14)x10^{10}$ as compared to $(4.38\pm0.53)x10^{9}$ in SmF. It should be noticed that spore and bacilli aggregates were found in the suspensions obtained from SSF but not in samples of SmF. This observation is suggestive of biofilm formation on the solid surface as compared to separate single cell behavior in SmF. Further biochemical studies in biofilm and quorum sensing phenomena will help to explain the major differences of *Bt* differentiation in such fermentation systems. Present results justify further research in order to take the most of much higher sporulation counts in SSF because it may render a more economical system with lower capital investment for commercial production of Bt spores.





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# Functional expression of a Bowman-Birk inhibitor from Tepary bean seeds and atypical interaction analysis with bovine chymotrypsin.

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Proteases are enzymes that cut peptide bonds of proteins and peptides. Because they are crucial in different physiological processes of various agencies, control of their activity can prevent possible cell damage. Protease inhibitors (PI's) are specific, and form stable complexes that lead the inactivation of the proteolytic activity of these enzymes. The PI's of Bowman-Birk family (BBI's) are found primarily in legume seeds, and their structure have two reactive sites for recognition of, generally, trypsin and chymotrypsin respectively. These inhibitors have proven efficiency and viability for cell modulation, thus have potential use for treatment of several types of cancer. However, for the inhibitor can be used for therapeutic purposes and / or biotechnological applications, is essential know the molecular basis responsible for variations in biological activity. One BBI identified in Tepary bean seeds (*Phaseolus acutifolius*), referred as TBPI, showed high stability by heat treatment and other extreme conditions, as well as, inhibition of proteolytic activity of insect pests (Campos *et al.,* 1997). Campos *et al.,* in 2004, reported the complete amino acid sequence of TBPI, which corresponds to a molecular mass of 9 kDa (Accession: P83311.1). Our working group has found that this inhibitor interacting with bovine chymotrypsin, under certain conditions, allowing proteolytic activity is maintained by forming a complex protease-inhibitor (PI) noncanonical. The aim of this work is to analyze the atypical interaction between TBPI and bovine chymotrypsin. For this, the gene sequence of TBPI was deduced, which was synthesized by the company GenScript®; the recombinant expression system TBPI (rTBPI) was constructed using the vector pET-19(+) (Novagen) and E. coli Shuffle T7 express strain (NEB). Different concentrations of inducer IPTG were tested, being more rTBPI expression in the soluble fraction of the cell extract using 40 μM IPTG. It was purified by chromatography rTBPI immobilized metal affinity (IMAC), followed by ion exchange chromatography, obtaining a yield of 5.2 mg / L culture. The $K_i$ value for trypsin was 7.8 x $10^{-9}$ M, and $K_i$ of 7.2 x $10^{-7}$ M was obteined for chymotrypsin. We used zymography analysis and formation kinetics, for canonical and noncanonical PI complexes. Results has proven that PI noncanonical complex is highly stable to temperature and presence of reducing as DTT. Understanding the structural and physicochemical basis of this atypical interaction is important to understand possible ways of escape of one of the defense mechanisms of plants by insects, including the provision of information to the study of the regulation of proteases in different organisms.

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# DESIGN OF A CHIMERIC GENE WITH ANTIMICROBIAL ACTIVITY DERIVED FROM *Moringa oleifera* Lam. AND ITS EXPRESSION IN *Chlamydomonas reinhardtiii*

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## ABSTRACT

Antimicrobial resistance to pathogenic microorganisms make evident the need to search new options to replace conventional antibiotics and antifungals. One option are the Plant Antimicrobial Peptides (PAMs) which are components of barrier defense system in response to pathogen attack (Nawrot, *et al.*, 2014). It has been reported that *Moringa oleifera* seeds possess antibacterial and antifungal (Suarez *et al.*, 2002; Gifoni *et al*., 2012) properties. The aim of this study was to design and optimize a chimeric gene that encoding a recombinant protein carrying an antibacterial activity peptide and express it in chloroplasts of *Chlamydomonas reinhardtii*. The chimeric gene called FLOGS was based on the sequence MO 2.1 reported in *M. oleifera* by Gassenschmidt *et al*., (1995). The chimera FLOGS designed includes flanking restrictions sites (*Nco*I) to carry out cloning, a histidine tag to detection and purification of the recombinant protein, a recognition site of thrombin protease to eliminate the N-terminal peptide FLO polypeptide, stop codon and ribosome binding site to the 3 'end. The FLOGS gene was cloned into the specific vectors p463 and p464 for expression in *C. reinhardtii* chloroplasts. The direction of insertion of FLOGS into the vectors was confirmed by PCR and sequencing. Positive clones were used to transformation of *C. reinhardtii* chloroplast by biolistic experiments. The selection of transplastomics lines will be identified by growth on TAP medium containing spectinomycin (150 mg/L) after 5-6 weeks, and corroboration will be performed by PCR and sequencing. Quantification of the recombinant protein will perform by ELISA and functionality will be verified by testing with pathogenic bacterial strains. On the other hand, the bioinformatics analysis of FLO peptide showed that has homology with the albumin 2S protein of *M. oleifera* seed, which has antifungal activity. So presumably the FLO peptide could present antifungal effects. The results of functionality will be displayed during the XXXI National Congress of Biochemistry.

**Keywords.** Chimeric gene, *Chlamydomonas reinhardtii*, *Moringa oleifera*, antibacterial peptide, gene expression in chloroplast.

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# Chemical crosslinking of nitrilase filaments for develop a stable and reusable biocatalyst.

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Nitrilases catalyze the direct conversion of nitriles to the corresponding carboxylic acids and ammonia. These enzymes are members of the nitrilase superfamily that is characterized by having a homodimeric block with a αββα sandwich and a conserved, catalytic triad of Glu-Lys-Cys (1). Microbial nitrilases are active only as oligomers that rearranged themselves into long regular helices of variable length and some of them have the ability to assemble into large filamentous structures (2). Nitrilases have been considered as great alternative to chemical catalysts, as they have proved to transform a variety of organic nitriles in a stereoselective or regioselective manner.

Previous results of our group showed that recombinant nitrilase from *Rhodococcus sp*. strain V51B hydrolyze both aromatic and aliphatic substrates with rather high efficiencies. Long helical filaments were generated by residue truncation in the C-terminal tail with a significantly increased catalytic activity and thermostability; notwithstanding the nitrilase filaments will need to be improved for biotechnological or industrial applications.

The proposed strategy is the immobilization of the nitrilase filaments by chemical crosslinking of enzyme aggregates or CLEA´s (3). In this procedure, the enzyme was precipitated from an aqueous solution by adding a water-miscible organic solvent. In a subsequent step the enzyme molecules were cross-linked with polyaldehyde, which was obtained by oxidation of dextran, and further irreversible linked by borohydride reduction. The cross-linked nitrilase aggregate preparation showed a final recovery activity of 100%. Nitrilase CLEA´s remained stable for almost 28 days at 4°C and can be reused for up to 5 cycles, with only an approximately 10% enzyme activity loss. Studies of temperature and solvent effects on enzyme activity are in progress.

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# Effect of vermicompost on the lettuce innocuity

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Keywords: Vermicompost, food safety and crop production.

Vermicompost (VC) application to soil has positive effects on crop yields because improves their physical, chemical and microbiological features. However, VC does not contain enough nutrients to supply entirely crop requirements. The nitrogen (N) from VC is high and has short term effects as result of mineralization process. The main challenges of organic fertilizers are to warranty the innocuity of final products, which must be free of pathogens as *Salmonella* and *E. coli* and heavy metals (Cd and Pb). The above in order to verify that VC does not contaminate the vegetal material and this does not affect to the consumer.

The objectives of present study were: I) physical, chemical and microbiological characterization of VC, soil and mixture of both substrates; II) evaluate the effect of VC application on production of lettuce, in order to compare between application of organic fertilizer in contrast with its fractional supply, through of agronomic assessment; and finally III) evaluate VC effect on lettuce safety, detecting chemical and microbiological innocuity indicators.

The results indicated that the physical and chemical features of soil improved putting-on VC at soil. In addition, VC fractional application increased by 26% in lettuce fresh weight and 14% for total weight (lettuce and root). Chemical fertilization overcame by 13 % and 11.9% in lettuce fresh weight and total weight, respectively. We detected soil and VC initial occurrence of *Salmonella* and *E. coli.* In the final step of crop development, only *Salmonella* survived at root and were absent in treatment with vermicompost. We did not detected helminth eggs and heavy metals.

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# Cytotoxic activity of *Thevetia peruviana* extract against cancer cell lines.

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Area: Biotechnology

In recent years, secondary plant metabolites (Phytochemicals) have been extensively investigated as a source of medicinal agents. *Thevetia peruviana* (also known as yellow oleander) it's a plant that belongs to the family *Apocynaceae* and it is frequently grown throughout the tropical and sub-tropical regions. All parts of the plant, particularly the seeds are poisonous due to the presence of cardiac glycosides or cardiac toxins, which act directly on the heart. Some toxic compounds that have been investigated in *T. peruviana* include: cardiac glycosides, thevetin A & B, thevetoxin, peruvoside, ruvoside and nerifolin.

In this work we decided to evaluate the cytotoxic activity of the methanolic extract from *T. peruviana* against human cancer cells, for which, the fruit was air dried at room temperature, powdered, and extracted by maceration at room temperature with methanol for 72h. The supernatants were filtered and evaporated with a rotary evaporator to obtain methanolic extracts. The tested cell lines were: colorectal adenocarcinoma (HT-29, ATCC® HTB-38™), large lung cell cancer (NCI-H460 [H460], ATCC® HTB-177™), prostate carcinoma (DU 145, ATCC® HTB-81™), breast adenocarcinoma (MCF7, ATCC® HTB-22™) and human fibroblast (CCL-116, Detroit-548) and african green monkey kidney (VERO, ATCC® CCL-81™) as non-tumorigenic cells lines. Cytotoxicity was assessed with the MTT assay. The concentration of the crude extract that killed 50% of the cells ($LD_{50}$) was calculated by minitab 17 software and all determinations were performed with quintuplicate. Doxorubicin, a known anti-cancer drug was used as positive control. In order to determinate the antimetastatic capabilities of the extract, adhesion and cell migration assay were performed by clonogenic and wound/healing assays. The colonies were counted by ImageJ software and the wound area was calculated using the Tscratch software.

Results from MTT assays showed that *T. peruviana* has an important cytotoxic activity against the cancer cell lines HTB-38, HTB-177, HTB-81 and HTB-22, with a $LD_{50}$ < 30 µg/ml. On the other hand, the human fibroblasts cells showed a $LD_{50}$ >1000 µg/ml. VERO cells $LD_{50}$ was around 60 µg/ml. Doxorubicin showed a $LD_{50}$ of ~10µg/ml for HTB-38 cell line. The clonogenic assays revealed that cancer cell lines have a survival fraction <30%, in contrast, the human fibroblast have ~85% of survival fraction and VERO cells ~60% of survival fraction. Wound and healing assays showed a delay in the wound closure time for colorectal and breast carcinomas when exposed to *T. peruviana* extract. For prostate and lung carcinomas the exposition to the extract caused the disaggregation of the cell monolayer, and human fibroblasts and VERO cells were unaffected by the extract. All this results suggest that the extract of *T. peruviana* fruit not only has an important cytotoxic activity, but also has selectivity against cancer cell lines, because the non-tumorigenic fibroblasts were unaffected on all the assays.

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# Evaluation of molecular and metabolic events during recombinant protein production in *Escherichia coli* with a thermo-inducible system

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The production of recombinant proteins of pharmaceutical or industrial interest in *Escherichia coli* has been extensively studied to find strategies to increase yields and reduce costs of production at large scale. The expression system induced by temperature is the most studied. The thermo-inducible systems do not require the addition of chemical inducers like IPTG, which may be expensive and toxic to cells and humans. Other advantage of this system is the low contamination risks, minimized by handling. However, the stress generated by the temperature increase used for the induction can often affect cell growth and to activate of bacterial heat shock response (HSR). These molecular events have been studied in separate contexts, but it converge in the activation of chaperones and proteases genes.

The principal aim of this study was to analyze the growth and production levels of the antigen ESAT-6 of *Mycobacterium tuberculosis* and some proteins associated with heat shock response in a recombinant *E. coli*, using an expression system induced by temperature. To achieve this goal, submerged culture of the recombinant strain *E. coli* ATCC 53606 (PDB + ESAT-6) in 250 mL shake flasks and 1.2 L bioreactors were carried out testing different culture media and heat induction strategies. Growth of the recombinant *E. coli* was followed by optical density (OD) at 600 nm. Organic acids production, glucose concentrations, Dissolved Oxygen Tension (DOT) and pH were quantified. The expression of proteins was determined by SDS-PAGE and Western blot. The protein aggregates were subjected to enzymatic degradation with proteinase K and denaturation with guanidinium chloride.

We found that growth of recombinant *E. coli* decreased in cultures at 39°C and 42°C compared to the cultures without induction (30°C), which can be attributed to the heat stress and metabolic burden. In shake flasks, glucose is not consumed completely; acetate was accumulated over time and the culture showed oxygen limitation. While in bioreactors, controlling the pH and the concentration of dissolved oxygen in the medium, glucose was consumed and the residual acetate began to be consumed at the end of the culture. Finally, we found that the recombinant protein ESAT-6 was trapped in inclusion bodies and chaperones like DnaK/J and GroEL/S showed a differential expression.

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# Overexpression of human cystatin C in *E. coli* and its purification; Isolation of recombinant protein that has been contaminated by nucleic acid after breaking the cells.

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Abstract:
Cysteine proteases (CPs) comprise a group of proteolytic enzymes that cleave the peptide bonds by the use of a cysteine residue at the catalytic site. Cystatins constitute a powerful regulatory system for endogenous CPs which are often secreted or leaking from the lysosomes of dying or diseased cells. One of the most studied protein inhibitors of CPs has been cystatin C. In this work, we present the overexpression and purification for recombinant human cystatin C. In the purification process the recombinant protein was contaminated by nucleic acid; this problem was solved by using anion exchange chromatography.
A cDNA encoding human cystatin C (cyst hum) was cloned into the pET-24(+) expression vector (cyst hum-pet 24) and then transformed into *E.coli* C3030H(DE3)SHuffle T7 Express lysY expression host. The recombinant human cystatin C (cyst Ch) was purified by a method that involved the following steps: breaking the cells, centrifuging, passing the soluble fraction by a gel filtration column (Superdex 200), equilibrated with 50 mM glycine, pH 9.3, separating the cyst Ch fraction in an anion exchange column (DEAE), equilibrated with 50 mM Tris, pH 8.5, and finally submitting the cyst Ch fraction to gel filtration in a Sephacryl S200 column, equilibrated with 50 mM phosphate, pH 7.4.
The spectroscopic and physicochemical characteristics (enzyme inhibition activity and molecular weight of 12282 Da by mass spectroscopy) of purified cyst Ch were verified. The near-ultraviolet absorption, circular dichroism and fluorescence emission spectra were all found to be similar to the corresponding spectra for human cystatin C previously reported. Other properties of cyst Ch were: dissociation equilibrium constant ($K_d$) of 24 nM (for the binding to inactivated chymopapain), estimated molecular weight of 13.2 KDa and 1.8 nm for the hydrodynamic radius (monomeric form), both analyzed by dynamic light scattering.
In conclusion, we have developed a method to isolate and purify recombinant human cystatin from overexpression in *E.coli*. After breaking the cells, a fraction containing cystatin contaminated with nucleic acid was resolved by anion exchange chromatography, and further purified by gel filtration chromatography.

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# "Study of the histidine kinase RetS over the expression of the small regulatory RNAs of the family *rsm* in *Azotobacter vinelandii*"

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The two-component regulatory systems (TCS) are type of signaling pathway that is widely distributed in prokaryotes, which are typically formed by a histidine kinase and its effector protein called response regulator. The TCS generate adaptive responses based on the detection of an environmental signal, subsequently activating a phosphorylation cascade that culminates in the transcriptional regulation of target genes of the system.

In *Azotobacter vinelandii* the ability to produce metabolites of industrial interest, as the intracellular polyester poly- β-hydroxybutyrate and the extracellular polysaccharide alginate, is controlled by the two-component regulatory system GacS/A. GacS is a histidine kinase located in the membrane, which on receipt of a stimulus suffers autophosphorylation and then phosphorylates its response regulator GacA, that acts as a transcriptional regulator of the metabolic pathways that produce alginate and PHB. The GacS/A TCS also controls the RsmA/Z System, a post-transcriptional regulation system, which in *A. vinelandii* has a large number of regulatory RNAs (seven of the RsmZ family and one of the RsmY family).

In *P. aeruginosa* there are two additional histidine kinases (RetS and LadS), other than GacS, that are proposed to control the activation of GacA. RetS and LadS has been shown to also regulate the *rsmZ* expression, RetS negatively and LadS positively. An orthologous gene of *retS* and an homologous protein of LadS in *A. vinelandii* were found. *retS* and *ladS* mutants of this bacteria show an increase in alginates synthesis, being more notable with *retS*, suggesting that unlike that reported in *P. aeruginosa*, both are negative regulators of this polymer synthesis. Since GacS/A control the synthesis of alginates by regulating directly the expression of the regulatory RNAs, we proposed to monitor the expression of this sRNAs in *retS* mutants by using transcriptional fusions *rsmZ/Y-gusA.*

Once obtained recombinant strains that carried the mutation of *retS* and the fusions of each one of the regulatory RNAs, the measurement of the effect of the mutation was performed by setting the β-glucuronidase activity encoded by the reporter gene *gusA.*

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# Determination of antimicrobial activity of *SPC13*, antimicrobial peptide isolated from the venom of *Scolopendra polymorpha*, against gram-negative bacteria

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**Keywords:** *Antimicrobial peptide, SPC13*, *S. polymorpha.*

**Introduction:** Antimicrobial peptides (AMPs) are an important part of innate immunity in vertebrates and invertebrates. Such molecules are cationic, amphipathic, low toxicity, broad spectrum, exert localized effect and induce little resistance in pathogenic microorganisms. Many AMPs have been isolated from bacteria, plants, skin of amphibians, and venoms from spiders, scorpions and centipedes[2].In particular, some AMPs have been found in the venom of centipede species from the genus *Scolopendromorpha*, such as peptides from *S. subspinipes mutilans* (2.5-4.4 kDa) with broad spectrum antimicrobial activity[1,4]. Recently, SPC13 was isolated from *S. polymorpha* venom by the electroelution method. SPC13 showed antimicrobial activity on *S. aureus* (ATCC 29213), its Minimum Inhibitory Concentration (MIC) was 9 µg/ml and produced 20.5% hemolysis. It showed a 98% of homology with histone H3 reported in *S. viridis* (GenkBank: DQ222181.1), according to a partial sequence analysis[5]. In this study, we tested both the antimicrobial activity and the MIC of *SPC13* against two Gram-negative bacteria. **Methods:** The purification and determination of antimicrobial activity of SPC13 were performed according to the protocol described by Rodriguez-Alejandro (2014)[1]. This AMP was tested against a Gram-positive strain (*S. aureus* ATCC 29213 used as a control) and two Gram-negatives (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853). Determination of MIC was performed by growth kinetics in the presence of SPC13 at different concentrations (48 to 192.5 µg/ml) according to the guidelines established by the Clinical Laboratory Standards Institute (CLSI)[3], bacterial growth by means of monitoring absorbance changes (595 nm) versus time of incubation and their respective controls of each inoculum. The MIC values obtained from active peptide were expressed as the mean plus standard error (mean ± SEM). The statistical level of significance taken for this analysis was $\alpha < 0.05$. **Results:** After purification of *SPC13*, antimicrobial activity tests were carried out by the agar diffusion method (ADM) where this peptide showed activity against *P. aeruginosa* at two different concentrations (1.5 and 3 µg/µl) forming inhibition halos of 6 and 8.7 mm in diameter respectively, whereas the MIC for this strain was 192.5 µg/ml. Two concentrations of SPC13 were tested on *E. coli* (3µg/µl and 6.6µg/µl) by the ADM, but none of them showed activity against this specific variant. Subsequently, two higher concentrations were used for the MIC assay (75 and 155 µg/ml), where SPC13 exhibited bacteriostatic activity on *E. coli* (ATCC 25922). **Conclusions:** *SPC13* showed dose-dependent antimicrobial activity against *P. aeruginosa* with an MIC of 192.5 µg/ml. Regarding *E. coli*, this peptide has bacteriostatic activity at the concentrations tested (75 and 155 µg/ml).

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# Characterization of lead compounds bioaccumulated by bacterial strains isolated from metal contaminated soil

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For many centuries, the mine industry has contributed to the development of Zacatecas economy. However, soil and water contamination with heavy metals is generated as a result of inadequate procedures and residues disposals with mineral materials that produce danger to human health and ecosystems. Because metals are not degradable; development of bioremediation techniques constitute a good option for restoration of these contaminated soils in Zacatecas. This work is focused on the characterization of lead compounds formed by seven lead-tolerant bacterial strains with MICs (Minimal Inhibitory Concentrations) between 4.5 and 7.3 mM of Pb $(NO_3)_2$. The lead bioaccumulation ability of this strains, determined by Atomic Absorption Spectrometry (AAS), revealed that accumulation in the isolates identified as *Staphylococcus hominis* (PbT-1 and PbT-3) was higher compared to those isolates identified as *Staphylococcus saprophyticus* (PbT-7) and several *Bacillus* species (PbT-2, PbT-4, PbT-5 and PbT-6). Moreover, the characterization of the lead compounds, carried out by Fourier Transform Infrared Spectroscopy (FT-IR) and X-Ray Diffraction (XRD) analyses, revealed an interaction of lead with phosphate and hydroxyl groups from bacterial cell wall and formation of hexagonal crystals of different inorganic lead phosphates such as lead hydroxyapatite, pyromorphite and other lead phosphates. Together, this results suggests that tolerance of this strains is related to a lead bioprecipitation mechanism on which production of inorganic phosphates plays an important role.

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# Isolation and identification of metal-tolerant filamentous fungi and their use in the synthesis of gold and silver nanoparticles

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Zacatecas is one of the main mining centers in Mexico, however, mining activities have generated a lot of waste deposited on the soil's surface causing serious disturbances to the environment and limiting the establishment of the vegetation, thus, affecting the biota and soil quality. The study of filamentous fungi diversity in soils contaminated with heavy metals provides an opportunity for new strategies in biotechnological processes involved in the metal nanoparticles (NPs) synthesis, bioremediation and metal recovery. This study shows the identification of two gold-tolerant and one silver-tolerant fungi strains isolated from metal-contaminated soils from Zacatecas. The morphological analysis and molecular identification by sequencing of ITSs from rRNAs genes, determined that this isolates belong to species of *Fusarium* and *Epicoccum* genera, whose MIC values (minimum inhibitory concentration) were 1.0 mM of $AgNO_3$ to *Fusarium* sp., 3.0 mM of $AuCl_3$ to *Fusarium* sp. and 2.0 mM of $AuCl_3$ for *Epicoccum nigrum*. Furthermore, the capacity of this fungal strains for synthesis of gold and silver NPs, was demonstrated by physicochemical analyses such as UV-Vis and Infrared (IR) Spectroscopy and X-Ray Diffraction (XRD). Thus, the contribution of this study was the establishment of a protocol for synthesis of silver and gold NPs and to the knowledge of the diversity of filamentous fungi in soils contaminated with heavy metals in the Zacatecas.

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# Isolation of bacteria from extreme environments from Baja California Sur with antibacterial activity against multidrug-resistant *Staphylococcus aureus*

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Key words: Extremophile bacteria, antibacterial activity, pathogenic bacteria.

The use of antibiotics against pathogens in medicine, agriculture and aquaculture has resulted in the development of bacteria with resistance to multiple drugs. It has been suggested that microorganisms from extreme environments are a novel source of antimicrobial compounds. The aim of this paper was to isolate and indentify bacteria from extreme environments with antibacterial activity against multidrug-resistant (MDR) *Staphylococcus aureus*. Bacteria were isolated from hyperarid environments (dunes), hypersaline environments (marine saltern) and environments with temperatures above 40 °C (volcanic fumarole and shallow marine hydrothermal vent) from Baja California Sur, México. Antibacterial activity against methicillin-resistant *S. aureus* (MRSA) was evaluated by broth microdilution assay and disc diffusion susceptibility test. Also minimal inhibitory concentration (MIC) was calculated and compared. Marine bacteria with anti-MRSA activity were identified by 16S rRNA. Antibacterial activity of anti-MRSA bacteria will be evaluate against other 17 MDR *S. aureus* with different resistance profiles. Finally, a multipassage resistance selection assay will be conducted with the highest anti-MRSA activity bacteria. A total of 584 bacteria were isolated: 247 from hypersaline environments, 167 from hyperarid environments and 170 from volcanic fumarole and shallow marine hydrothermal vents. In the broth microdilution test, 23 of all isolates inhibit the growth of MRSA by 85% or greater, while 18 isolates inhibited the growth by 100%. Among them, *Brevibacillus laterosporus* (isolated from lichen near to dunes, a hyperarid environment), *Bacillus circulans* and *Virgibacillus proomi* (both isolated from shallow volcanic hydrothermal vents) were identified. Interesting, in the disc diffusion test *B. laterosporus* inhibited the growth of MRSA by 70% of the positive control (gentamicin, 100%). The experimental results showed that exist culturable bacteria from extreme environments in Baja California Sur, México, with antibacterial activity against multi-drug resistant *S. aureus*.

Area: Biotechnology

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# Isolation and characterization of levanase of *Clavibacter michiganensis subsp michiganensis.*

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7772293533INTRODUCTION.
Functional foods are new products of the food industry that have great commercial impact, because they provide a health benefit, and also they contribute to basic nutrition (1).
The active biological components that are present in functional foods are called prebiotics, which are food ingredients that are resistant to digestion in the stomach and the small intestine and may reach the colon where they are selectively fermented by a specific microbial group.
Among the most studied probiotics are inulin-type fructans where predominate linkages β (2-1), and levan-type fructans that present linkages β (2-6). In the same way, fructooligosaccarides (FOS) are polymers that have a degree of polymerization between 2 and 10 units of fructose, FOS of inuline have the higher prebiotic activity, however, FOS derived of bacterial levans have not been well characterized (2). An alternative to produces FOS type levan will be through levan hydrolysis with a high molecular weight and linkange β (2-6) with endo levanases.

Our objective is to isolate the gene encoding for the levanase of *Clavibacter michiganensis subsp michiganensis.*

METHODOLOGY. Primers were designed with XhoI and NcoI restriction sites. In order to isolate the levanase gene, chromosomal DNA from *Clavibacter michiganensis subsp michiganensis* was purified and used as a template with Taq polymerase to amplify a PCR fragment, this product was cloned with pET28 vector in *E. coli* DH5α. The correct construction of the plasmid was confirmed by sequence analysis of single DNA strans from the insert. Positive transformants were selected and the optimal expression level was performed.

RESULTS AND DISCUSION.
The analysis of the levanase aminoacid sequence revealed the non-presence of a signal peptide that was predicted with (Signal P 4.1). Blast analysis of levanase from *Clavibacter michiganensis subsp michiganensis* showed that it has an identity with *Arthrobacter sp. PAO19* (47%), *Pseudoalteromonas haloplanktis* (47%) and *Microbacterium trichothecenolyticum* (50%). Specifically in the N-terminal domain of glycosyl transferase family 32, which forms a five bladed β propeller structure, it has the C-terminal domain of glycosyl hydrolase family 32, it forms a β sandwich module. Levanase has a molecular weight of 59 kDa and its isoelectric point is 4.63.
The gene encoding for the levanase was amplified by PCR using specific primers, The product of around 1641 bp amplified was cloned and sequenced.

CONCLUSION.
Levanase of 59 kDa and 547 aminoacids has a high identity with the glycosil hydrolase family in the active site. Conditions to express the protein had been stablished.

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# Implementation of a new molecular tool to generate multiple mutants *in Azotobacter vinelandii*

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*Azotobacter vinelandii* is a bacterium with biotechnological interest due to its capacity to produce secondary metabolites as PHB and alginates. PHB is a biodegradable and biocompatible polymer with similar proprieties to the plastics derived from petrochemicals. The alginate is a heteropolymer with gelling capacity which can be used in the food and pharmaceutical industry as a thickener or gelling[(1)].

The production of both polymers in *A. vinelandii* has been vastly studied, the polymers are co-regulated by the GacS/A two component system and the Rsm post-transcriptional regulatory system. The *rsm* system possess nine isoforms of small RNA regulators (sRNAs), encoded by different genes, thus to study the system is necessary to generate multiple mutants. However to generate multiple mutants would be necessary to have several useful markers of resistance. In our model are useful only four resistance markers thus became necessary implement a technical that allows eliminating the multiple genes without requiring resistance markers.

Site-specific recombination of flippase systems allow excision and recycling selection markers that contained FRT sequences, this system has been reported in *Burkholderia pseudomallei* [(2)] where by inducing of rhamnose the recombinase recognizes the FRT sequences cleaves the marker cassette that can use the same marker for following mutation. Using a modified FRT system in *A. vinelandii* has been made the first mutation in one gene of the Rsm famlily.

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# IDENTIFICATION AND CHARACTERIZATION OF HEAVY METAL TOLERANT ENDOPHYTE FUNGI FROM *Acacia farneciana*

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Mining is one of the most important economic activities of Mexico due to high exploitation of metal resources, Mines were exploited during the XVIII and XIX centuries in the zone of Huautla, Morelos (Mussali, 2013). It has been found that the main components of the tails are lead (Pb), copper (Cu), zinc (Zn) and arsenic (As). Phytoremediation offers more benefits than any of the other conventional technologies to recover the soil. One of the most popular plants in the zone of Huautla is *Acacia farneciana* that is considered as a heavy metal hyperaccumulator. Also it can help to control soil erosion and helps to increase soil fertility though symbiotic relationships with different microorganisms as mycorrhizas and nitrogen fixing bacteria (Santoyo, 2016). The use of fungi for contaminated soil recovery is called fungiremediation or mycoremediation, most of isolated endophyte fungi belong to phylum Ascomycota and Basidiomycota. In this work we present the isolation and characterization of five endophyte fungal strains isolated form the roots of *Acacia farneciana*; we have analyzed their growth different concentrations of heavy metals (Cu, Zn and Pb) in a range of 50 to 700 p.p.m. Four of them are capable of growing at 700 p.p.m. of Zn. The other one up to 150 p.p.m. Regarding Cu one was able to grow up to 50 p.p.m. The others grew at intervals that oscillate between 50 and 250 p.p.m. In the case of Pb four were capable of growing from 50 to 350p.p.m. and one was able to grow at 500 p.p.m. On average the tails of Huautla contain 2,413 p.p.m. Of bioavailable Zn, exceeding the standard set by Cleanup Standards for Contaminanted New Yersey Department of Environmental Protection (1996). 273 p.p.m. Which exceeds the maximum allowable limit by SEMARNAT and 25.5 p.p.m. Of Cu which support the results obtained by Velasco et al (2004) and semarnat (2005). Other three fungi are now in a process of evaluation. The characterization of these fungi, can have a big potential in bioremediation for which we consider it can be important the phylogenetic characterization.

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# EVALUATION OF BACTERIAL ENDOPHYTE STRAINS OF LEGUMES AS PLANT GROWTH PROMOTING RHIZOBACTERIA

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**Keywords:** Endophyte, PGPR, nodules, legumes.

One of the principal environmental problems in the actuality is the indiscriminate use of inorganic inputs. Mainly the use of inorganic fertilizers has altered in a significant way the quality of the soil and generated an ecological out balance. Different options have been proposed to decrease the use of chemical products, an alternative is the use of plant growth promoting rhizobacteria (PGPR). There exists a large number of PGPR for example: *Pseudomonas, Burkholderia, Bacillus, Azospirillum, Herbaspirilim, Enterobacter, Azotobacter*, among others (Karakurty Aslantas, 2010). In this respect, legumes are very interesting since these plants have ability to establish symbiosis with potential PGPR bacteria. So this work is focused to evaluate 23 isolates from nodules of legumes with potential to fix nitrogen, solubilize phosphate, production of indolacetic acid (AIA) and produce extracellular enzymes (chitinases), as well as the effects in the germination in tomato. From all the isolates, 10 showed phosphate solubilization properties and production of AIA, but only 3 were positive for the production of chitinases, The 10 isolates showed effect in the germination of tomato seeds. These strains are potential candidate for PGPR, also those strains producers of quitinases are candidates as biocontrol agents against fungi, whose cell wall is composed of this polysaccharide.

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# Characterization of microorganisms with biotechnological potential isolated from sites contaminated with heavy metals

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**Introduction.** The mining district of Guanajuato has generated large amount of solid waste (tailings) with high quantities of heavy metals such as Ag, Mn, Zn, Pb, Cd and As. These residues cause heavy metal contamination in the soil, subsoil, air and water, impacting the environment, causing biodiversity loss [1]. Heavy Metal environment select the presence of microorganisms that have been adapted to extreme environments by present heavy metal resistance mechanisms. This work focuses on the search for other biotechnological applications (in addition to resistance to heavy metals), where it can be useful the whole cell, enzymes or metabolites produced by it. **Objective:** Isolation and identification of heavy metal resistance microorganism from tailings of the mining district of Guanajuato, with potential biotechnological applications. **Methodology:** a) Sampling sites contaminated with heavy metals; b) Isolation of microorganisms with potential biotechnological applications isolated from the samples growing on selective conditions. c) Identification of microorganisms by biochemical and molecular methods. **Results and discussion:** Sampling was performed in different mining tailings of Guanajuato : “La Valenciana” (1999, 2015), “Noria Alta” (2016), “Nieto Piña” (2016) and “Monte de San Nicolás” (2016). The microbiological analysis was performed for each one of the samples. From “La Valenciana”, was recovered strain M11, which was preserved in glycerol 50% at -20 °C for 16 years, proving that still retains its high resistance to silver nitrate, and using molecular techniques was identified as *Nocardia sp.* From the last sampling on “La Valenciana” (2015) was obtained another strain collection, when 10 isolates were recovered, which have characteristics of *actinomycetes*, whose extracts will be analyzed to determine their effects. In this regards, various working groups have reported the isolation of *actinomycetes* resistant to zinc, lead or nickel from mine tailings of zinc, lead or nickel, some of which have been classified as new species in its genus. **Conclusion:** the sites contaminated with heavy metals, represent a unique opportunity to study microorganisms that have been adapted to extreme environments, which can present novel microbial processes.

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# PARTIAL PURIFICATION AND CHARACTERIZATION OF MILK CLOTTING ENZYME FROM *Moringa oleifera* Lam.

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**Background:** Chymiosin is the main enzymatic component used in milk-clotting due to its high specificity for the κ-casein and its low proteolytic activity. However, the increasing production of cheese, reduced supply of rennet and some ethical questions has advertised the research of alternatives to calf rennet. Microbial sources has been employed as substitutes, however natural rennet extracted from plant has reached increasing attention. *Moringa oleifera* has been object of research for this purpose, there are only two studies that reports the activity of milk clotting enzymes (MCE) in flowers and seeds extracts, but results on the presence of MCE are inconsistent. There are few reports about its application on different types of milk including the soy milk. **Aim:** Evaluate the potential coagulant and caseinolytic capacity of extracts of *M. oleifera* flowers, leaves and seeds on different types of milk. **Methods:** *M. oleifera* seeds, flowers and leaves were recollected from Lombardia, Michoacan crops. MCE were extracted by maceration under ultrasonic bath, later milk clotting activity (MCA) was determined using different substrate concentrations of skim and soy milk powders. Proteolytic activity (PA) of the extracts were determined spectrophotometrically and casein hydrolysis were evaluated by SDS–PAGE. Proteases involved in MCA and PA were identified by zymography and finally proteins were precipitated with $(NH_4)_2SO_4$. **Results:** No reports are known about the application of *M. oleifera* extract on soy-milk coagulation. Seed extract coagulated both skim and soy milks within a suitable time using low substrate concentrations, while flowers and leaves extracts are not good sources of MCE. Otherwise, the PA and hydrolisis of casein showed that seed extract promoted an extensive cleavage of casein similar to renin (positive control) compared with flowers and leaves extracts. Electrophoretic pattern analysis showed proteins from seed extract of 19 and 32-KDa were the most abundant compared with flower and leaves which have less proportion. On the other hand, the fraction precipitated with (40-70%) $(NH_4)_2SO_4$ has higher PA and MCA compared with the crude extract. **Conclusion:** Seed extract seems to be a potential candidate as calf rennet substitute to develop a soy cheese.

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# Study of the genes involved in the biosynthesis of Cyclodipeptides; cyclo(L-Pro-L-Tyr), cyclo(L-Pro-L-Phe) and cyclo(L-Pro-L-Val) from *Pseudomonas aeruginosa* PAO1.

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Different studies have reported that the cyclodipeptides (CDPs) have a great bioactive potential; presenting antibacterial and antifungal biological activities, they participate in quorum sensing, transkingdom communication, including cytotoxic activity. *Pseudomonas aeruginosa* PAO1 a Gram negative bacillus, has been reported to produce three cyclodipeptides; cyclo(L-Pro-L-Val), cyclo(L-Pro-L-Phe) and cyclo(L-Pro-L-Tyr). It had been previously described that cyclodipeptides could be intermediate products of the degradation of proteins, but currently two enzymatic systems that can synthesize cyclodipeptides have been described: first of them is catalyzed by no ribosomal peptide synthases (NRPS) and the second system is catalyzed by ciclodipeptid synthases (CDPS). It is suggested that the system for the synthesis of cyclodipeptides in *P. aeruginosa* PAO1 is through the system of NRPS. A protein Blast that employed AusA (*S. aureus*) as probe identified thirty-six putative NRPS in *P. aeruginosa. It has also been reported* in our work group that the cyclodipeptides of *P. aeruginosa* have cytotoxic activity. Therefore, in this study we identified nine genes as NRPS which were selected by high identity score and low E- values using an in silico analysis. This represents a possible network of genes involved in the synthesis of CDPs. We are studying the double mutants phenotypes of *P. aeruginosa* PAO1 for the NRPS genes by selection of bacterial confrontation trials and chemical analysis of the polar supernatant extracts to determine the compounds involved.

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# Effect of hyperoxidising atmosphere on UVB radiation resistance and its relationship with the activity of the enzyme Gpx in conidia of two strains *I. fumosorosea.*

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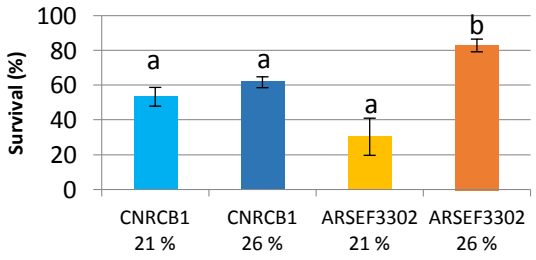
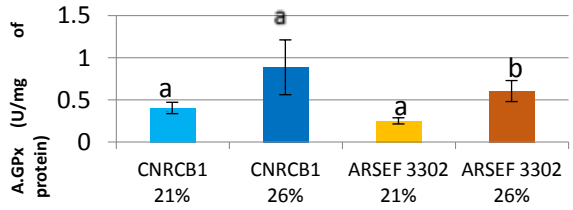
**Introduction.** *Isaria fumosorosea* is used in biological control programs, it is exposed to UVB radiation which stimulates the production of reactive oxygen species (ROS). In response, fungi use different antioxidant defense systems like enzyme glutathione peroxidase (GPx) [1]. On the other hand, it has been observed, for two strains of *I. fumosorosea*, that when they were exposed to an atmosphere rich in oxygen (26% $O_2$), this has led to a phenomenon known as cross-protection which causes an improvement in the quality of conidia. The aim of this study was to determine the effect of an oxygen-rich atmosphere (26% $O_2$) on resistance of conidia of two strains of *I. fumosorosea* exposed to UVB radiation and the activity of the antioxidant enzyme glutathione peroxidase.

**Methodology.** Were studied the strains CNRCB1 and ARSEF 3302 of *I. fumosorosea*. Cultivation and application of the oxygen-rich atmosphere (26% $O_2$) was performed based in Miranda Hernández *et al.,*[2]. The conidia were harvested at 156 h of culture and broke into a cell disruptor, immediately the enzymatic activity of glutathione peroxidase (A.GPx) was measure according to the instructions of Glutathione Peroxidase Assay Kit (CAYMAN). At the same time the soluble protein concentration by the Bradford method was measured, the reaction was measured at 595 nm. For the test resistance to UV-B radiation, 300 conidia were inoculated in Petri dishes which were subjected to 14 $kJm^{-2}$.

**Results.** In the test of resistance to UVB radiation(14 $kJm^{-2}$), it was found that the conidia of ARSEF 3302 obtained with the enriched atmosphere (26% $O_2$), increased their resistance to this radiation, improved by 50% the conidia resistance to UVB radiation compared to the survival of conidia obtained from 21% $O_2$. In contrast, the conidia of CNRCB1 showed no difference in survival in any of the treatments studied (Figure 1).




**1. Survival (%)** of conidia of *I. fumosorosea* to UV-B radiation (14 kJ / ned with different concentrations of oxygen in the atmosphere with CNR ns (21% ■, 26% ■) and ARSEF 3302 (21% ■ y 26% ■). Bars with different le significant differences (Student's t-test p<0.05)




**2. GPx activity** in *I. fumosorosea* conidia obtained with diffe en concentrations in the atmosphere with CNRCB1 strains (21 ■) and ARSEF 3302 (21% ■ y 26% ■). Bars with different le significant differences (Student's t-test p<0.05)

According with the results, an increase in A.GPx could be related with an increase in the resistance to UVB radiation in conidia of *I. fumosorosea* (ARSEF 3302, while for CNRCB1 no differences were found in A.GPx in any of the treatments tested (Figure 2), suggesting that exposure to an atmosphere enriched treatment at two different strains responses propitiated different to activity level of the antioxidant enzyme GPx which may be involved in increasing the resistance of UV-B radiation.

**Conclusions**. The oxygen-rich atmosphere (26%) favors an increase in the resistance to UV-B radiation in conidia of one of the two strains studied *I. fumosorosea*. This increase may be related, at least in part, to the activity of the antioxidant enzyme GPx. **Acknowledgement:** SEP-PROMEP, folio: UAM-PTC-562

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# Expression of amebic chitinase (*Eh*CHT1) on the surface of *Escherichia coli*

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The display of proteins on the surface of bacteria is regarded as an attractive approach for the production of functionally active enzymes anchored to the cell membrane [1, 2]. Gene fusion of a signal peptide with an anchor protein (*e.g.*, Lpp-OmpA) has become a successful technique to target proteins to the *Escherichia coli* outer membrane [1-4]. The amebic chitinase (*Eh*CHT1) is an important glycosyl hydrolase for the life cycle of the intestinal protozoan *Entamoeba histolytica*, the causative agent of human amebiasis. Interestingly, *Eh*CHT1 has structural features that make it valuable for biotechnological purposes: stable at a wide pH range (5.0 to 8.5) and broad thermal stability (up to 50 °C) [5], which can be explored in alternative processes for biodegradation of chitin. Here, we present the results of the functional expression of the enzyme catalytic domain (*Eh*CHT1c) on the surface of *Escherichia coli* BL21(DE3). The *Eh*CHT1c sequence was inserted in-frame with the Lpp-OmpA cassette, which controlled by the T7 RNA polymerase promoter. The expression of Lpp-OmpA-*Eh*CHT1 on the bacterial surface was demonstrated by subcellular fractionation and immunodetection assay. The physiological outcome of overexpression on the bacterial surface was assessed by determining the specific growth rate and the deleterious effect on cell survival. The biotechnological potential of bacterial cells displaying the amebic chitinase was evaluated by a fluorometric assay, using the synthetic compound 4-methylumbelliferyl-β-D-triacetylchitotrioside as substrate. Our results have shown that the catalytic domain of *Eh*CHT1 expressed on the surface of *E. coli* is functionally active. Currently, the catalytic activity is being evaluated on other substrates, including chitin and chitosan.

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# Binding Calorimetric Study of a Stabilized Human Cystatin C and Chymopapain

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Isothermal titration calorimetry (ITC) is a technique used to characterize the binding thermodynamics of any molecular complex by measuring directly the heats of binding during a titration. For this work the gene of the L47C-G69C human cystatin C was constructed, and the variant was expressed and purified, which according to Nilsson *et al.*[1] has and additional disulfide bridge that prevents domain swapping oligomerization at high protein concentrations, which are required in ITC experiments. The presence of this new disulfide bond was confirmed using MALDI-TOF mass spectrometry by comparing the molecular mass of the intact protein and the unfolded protein with reduced and carboxymethylated disulfides. According to the ITC results the enzyme-inhibitor complex possess a moderately high affinity ($K_b$ = 4.8 x $10^6$) and the binding process is enthalpically directed ($\Delta H_b$= -42.7 kJ/mol) with an unfavorable binding entropy, at 25 °C and pH 7.0.

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# *In vitro* propagation of pineapple (*Ananas comosus* (L.) Merr.) cv. 'Smooth Cayenne'

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## ABSTRACT

The pineapple, *Ananas comosus* (L.) Merr., is native to the South American Tropics, now is widely grown throughout the tropics and subtropics. The pineapple, is a crop of great commercial importance due to the high demand for its fruit. Although many methods have been applied in pineapple micropropagation, our study provides an efficient, simple, rapid and reproducible micropropagation system. As source of explant on shoot multiplication were used plantlets established *in vitro* of pineapple *A. comosus* L. Merr. Cv. Smooth Cayenne. The explants were cultured in culture vessels containing liquid MS medium with 3% sucrose (w / v) and supplemented with different concentrations (1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 mg. L-1) of benzyladenine (BA) or 2-isopentyl adenine (2iP). All cultures were maintained for 8 wk under light for multiplication of shoots. For induction of roots, seedlings were grown for 4 wk on MS medium without plant growth regulators (RCVs), solidified with 0.8% (w / v) agar supplemented with activated charcoal (0.2% w / v). In both steps the cultures were maintained under continuous light. In vitro propagation of shoots was successful from pineapple explants, which responded quickly in liquid culture MS medium, obtaining a significantly number of shoots. Higher multiplication rates for *A. comosus* L. were obtained with 4.0 $mg.L^{-1}$ BA, 69 shoots per explant. Pineapple cultivation in liquid medium favors the multiplication of shoots. The longest of shoots (14.09 cm), occurs at low concentrations of cytokinins, the higher the concentration of cytokinins length of shoots decreases considerably, yielding a greater number of shoots. Proliferated shoots produced roots with maximum frequency on MS medium without growth regulator at 6 wk. Plants rooted in the same liquid medium, however, on MS medium solid with activated charcoal are obtained more roots, longer and vigorous, the plants obtained from *in vitro* culture require a good root system (higher number of roots) to succeed in transplantation and adaptation to greenhouse conditions. All the plants were successfully acclimatized, with respect to percent survival in greenhouse was 100%. In this sense, regenerated plants had a normal appearance, very similar to plants growing in field.

# Inulinases as an alternative to the use of agave and its wastes to produce fructose and fructooligosaccharides.

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Inulinase (2,1-β-D-fructan fructanohydrolase, EC 3.2.1.7) is an enzyme of importance due to its ability to hydrolyze inulin, a non digestible carbohydrate fructan polymer consisting of of β-(2,1)-D-Fructofuranosyl-D-fructofuranosides link. The enzyme releases fructose and/or fructo-oligosaccharides as products, which are widely applied in medicine and the foodstuff [1]. Mexico produces large amounts of *Agave tequilana* for tequila production. Leaves of this plant contains about 20% of inulin, that can be used for transformation purposes. However, unlike chicory inulin, inulin of agave has β-(2-1) and β-(2-6) linkages in a branching molecule. Its use is limited by lack of enzymes capable of hydrolyzing these polymers.

We isolated a strain (ISO3) able to utilize agave inulin as carbon source. ITS1 and ITS4 sequence analysis identified the strain as *Kluyveromyces marxianus*. The strain was cultivated in minimal medium with different carbon source (inulin of agave, fructo-oligosaccharides, saccharose, fructose, glucose). Maximum inulinase activity was obtained by culturing *K. marxianus* ISO3 on inulin. The characterization of the activity revealed that it is an exo-inulinase.

The enzyme was purified by using anion exchange and molecular exclusion chromatography. It shows a MW is ~100 kDa by SDS-PAGE and zymography. The enzyme activity was found to be 8 U/mL with a temperature and pH optima of 50 ºC and 5.5, respectively. This enzyme represents a first step into the biotechnological transformation of agave inulin.

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# Genomic sequencing and characterization of the metabolic pathway involved in chloranilic acid degradation on *Herbaspirillum* sp. TQ07

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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 elucidate in detail the metabolic capacity that certain microorganisms have for remove toxic compounds by biodegradation. In this work, the genome of *Herbaspirillum sp.* strain TQ07 was completely sequenced. This strain use 2,5-dichloro-3,6-dihydroxybenzo-1,4-quinone (chloranilic acid, CA) as only source of carbon and energy and 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 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 (of which 41 were longer than 1 kb), with 60.31 GC%. Genomic annotation was performed via RAST using the genus *Herbaspirillum* as reference, resulting in 4571 annotated features; two of these contigs contain putative genes related to CA degradation. The strain TQ07 spontaneously loses the ability to degrade CA when grown in rich media; this phenotype is attributed to a recombination mechanism that causes the loss of an 80 Kb genomic island. Also we found, that only *Burkholderia cenocepacia* strain DDS 22E -1 has a set of homologous genes similar in number, orientation and genomic distribution of 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. Additionally, the genes involved in the degradation of CA are not present in other strains of the genus *Herbaspirillum* which reinforces the hypothesis that they were acquired by TQ07 strain through a horizontal transfer. Bioinformatics analyses are in develop to identify another genes involved in the degradation of CA like dioxygenases. In fact, in one of the contigs related to CA degradation we found a catechol-2,3-dioxygenase pathway.

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# Degradation of pyrene and phenanthrene in hypersaline conditions by Aspergillus caesiellus H1 halophyla strain.

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## Abstract

Polycyclic aromatic hydrocarbons (PAHs) are fat-soluble substances that are formed as products of combustion and waste oil coal processing. They are among the contaminants most biological impact, because of the carcinogenic and mutagenic effects that cause in living systems. In recent years it has increased interest in the use of biotechnologies aimed at restoring contaminated environments petroleum or petroleum products, which complement traditional chemical and physical methods. By thus seems bioremediation technique based on the use of microorganisms to degrade xenobiotic, including PAHs present in oil and other fuels. Fungi have the ability to transform and degrade organic compounds to simpler compounds, which offers them an undeniable potential for use in processes such as these. Many of the HPA contaminated environments are further characterized by being salted or hypersaline, which constitutes an additional challenge for bioremediation strategies, since there is little information on halophilic microorganisms capable of degrading HPA. The aim of the paper is to analyze the degradation of pyrene and phenanthrene and removal from industrial wastewater in hypersaline conditions (2M NaCl) by Aspergillus caesiellus H1 strain halophyla. Together studied the enzyme profiles of laccases, peroxidases and esterases, related HPA catabolism and biotechnological potentials. Waiting result in expression of oxidative enzymes in different crops with phenanthrene and pyrene presence of hypersaline conditions.

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# IDENTIFICATION AND ISOLATION OF PROTEINACEOUS COMPOUNDS WITH ANTIMICROBIAL ACTIVITY IN THE *Scolopendra viridis* VENOM

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Antimicrobial peptides (AMPs) 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. Some AMPSs have already entered clinical trials, for example: Magainin, a peptide identified from *Xenopus laevis,* has been used to treat diabetic feet ulcers (Rossi *et al*., 2008). Many AMPs from arthropods such as insects and scorpions have been thoroughly studied (Muller *et al*., 2008); in the venom of the centipede *S. subspinipes mutilans*, there are reports of several peptides with both antibacterial and antifungal activities, namely Scolopendrin 1 (Wenhua *et al.,* 2006), Scolopin 1 and Scolopin 2 (Peng *et al.,* 2009). *Scolopendra viridis* venom extraction was performed by mechanical stimulation of the forcipules; venom was quantified by the Lowry method. Antimicrobial activity was evaluated by direct agar diffusion by SDS-PAGE, according to León *et al*. (2005); briefly, venom was separated by 16% SDS-PAGE, 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 (5mg/ml), 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 control, PBS as negative control and 5mg of the active peptides/proteins; red blood cells were incubated for an hour with controls or peptides/proteins, then optical density was measured at 415 nm and the absorbance of the supernatant of the positive control was considered as 100% hemolysis. We found 4 peptides 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.

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# "LYTIC ACTIVITY OF MELITTIN ON *Neochloris oleoabundans* (CHLOROPHYTA) FOR ENHANCE NEUTRAL LIPID EXTRACTION"

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One of the limitations to the economic competitiveness of biofuels from microalgae is the inability of their cells to excrete neutral lipids, the technology used must involve the breaking of membrane structures for release the product of interest, therefore is important find other alternatives in the cell disruption process of microalgae *Neochloris oleoabundans* that can combat this problem. Melittin is a water-soluble cationic amphipathic alpha-helical peptide derived from the venom of the honeybee. The basis of melittin action is a physical and chemical disruption of membrane structures that leads to cell lysis. Although its lytic activity against various cell types have been reported, there is no information about its behaviour on microalgae.

**Objectives:** Evaluate the activity of lytic peptide melittin on cells of the microalgae *N. oleoabundans* by qualitative evidence.

**Methodology:** UTEX 1185 (Culture collection of algae at The University of Texas at Austin) was obtained; this flask was conditioned in Bristol medium without any stress. The ability of melittin to cause lysis was assessed using two types of treatments and three controls, which were: (10 min, 20 min, 30 min.) peptide melittin three times incubation and subsequent sonication to previous treatment with the peptide. Controls were: no treatment microalgae, negative control and negative control peptide melittin sonication. For all experiments, cells were adjusted microalgae to an optical density of 0.1. In the first treatment the peptide melittin, at 28 °C, 1 ml of cells was incubated with peptide 50µl at a concentration of 50 ug for 10, 20 and 30 min. with an agitation of 500 rpm. In the second treatment the same methodology was applied after adding to each condition, the sonication with 6 cycles of 10 sec. and 50% amplitude. Subsequently microscopic observations were made with a 40x magnification for each condition in order to check the activity of melittin peptide and sonication on microalgae. Results: conditioning the strain UTEX1185 to flask level was achieved and from these cell culture experiments corresponding to the first stage were performed. In microscopic observations was observed in all fields complete deformation of the cells under all incubation times peptide with and without sonication, therefore, in the next step lower peptide concentrations prove to perform step quantitative and to assess the precise behavior of this before microalgae.

**Conclusion:** Pretreatment of cell lysis by the lytic peptide melittin has activity on cells of the strain *Neochloris oleoabundans,* which is encouraging to continue with the next stages of the project.

# Cytotoxic Effects and Apoptosis Induction by Defensine Gamma Thionin (*Capsicum chinense*) and Butyrate in Colon Cancer Cell Lines

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Gamma thionin (GT) from *C. chinense* is a plant antimicrobial peptide which belongs to β-defensin family. This defensin is cytotoxic to cancer cells from solid tumors and leukemia. However, concentrations at which exerts cytotoxic effects are high ($IC_{50}$ >100 µg/ml). On the other hand, butyrate is a short chain fatty acid produced by fermentation of dietary fiber in the intestine, which induces cell cycle arrest and promotes apoptosis in colon cancer cells, whose effects may be potentiated by other molecules. The aim of this study was to evaluate the cytotoxic effect of combined treatment of GT and butyrate on colon cancer cell lines Caco-2 and HCT116. Cells were pretreated with 20 µg/ml of GT for 24 h, and then butyrate was added to a final concentration of 5 mM for HCT116 cells and 50 mM for Caco-2 cells. The combined treatment was incubated for 48 h with the cells. Butyrate inhibited Caco-2 cell viability ~60%, while GT only ~30%, and the combination ~65%. Interestingly, despite the combined treatment did not show a significant decrease in the number of viable cells as compared with butyrate treatment alone, it showed a significant increase in the amount of dead cells analyzed by trypan blue exclusion assays. In the same way, HCT116 cells treated with butyrate showed a ~20% reduction in viability but GT did not affect it; however, the combined treatment increased this effect (~35%). Also, a significant increase in the amount of death cells was detected. Subsequently the rate of apoptosis and necrosis was analyzed by flow cytometry using Annexin V and 7AAD. Induction of apoptosis on Caco-2 cells was detected after 6 h of the combined treatment, and after 24 h ~35% of cells were apoptotic. Butyrate alone induced a 10% of apoptosis and GT 20% of apoptosis at the same time of treatment. Thus, we can conclude that the combined treatment of GT and butyrate increases apoptosis through an additive effect in Caco-2 cells. In relation to HCT116 cells, apoptosis was observed until 24 h of the combined treatment (10%). In butyrate treated cells 20% of apoptosis was detected and in GT treated cells apoptosis was not detected. Therefore, the increase in cell death generated by the treatment with GT and butyrate probably follows a different pathway than apoptosis.

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# Maize differential tolerance on water stress conditions: Contribution of soluble solutes and dehydrins.

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Drought is one abiotic limiting factors in agriculture, causing a reduction of crop growth, biomass production and yield. Maize lines with tolerance to drought respond to water stress changing their physiology and metabolism. Some of these responses include a decrease in the relative water content and in the osmotic potential, as well as the accumulation of solutes such as proline, several sugars and alterations in protein content. Maize plants have the ability to generate adjustments that allow them to adapt and survive under water deficit. This work analyzed the effect of water deficit on the accumulation of solutes and protein changes mainly dehydrins, in two maize lines L-14 (tolerant) and L13 (susceptible). Plants with 7 weeks after emergence were subjected to gradual water supply deficit of 50% by one week. The results showed that solute accumulation in the leaves from line L14 at different levels water deficit resulted in an increased accumulation of proline, sugars and DHN1. On the other hand the solute accumulation and DHN1 in leaves in line L13 was lower. Changes in dehydrin content were also observed with heights values for line L14. Leaves of plants from line L14 subject to water deficit showed a decrease in the relative water content (RWC) to 90 %, versus Line 13 to 70 % and of the osmotic potential (ψs) lower from line 14 versus L13, and also an increase in the accumulation of solutes: proline, glucose, fructose, sucrose and trehalose, and in the soluble protein content. Our data support the hypothesis that the solute accumulation and DHN1, by its protective role, contributes to an improved tolerance to drought osmotic adjusments in plants of the line 14.

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# Cell surface display of proteins on filamentous fungi

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Cell surface protein display consists in the co-translational fusion of a functional protein (passenger) to an anchor one, usually a cell-wall resident protein. Protein display approaches have been useful to set up new catalytic activities on the cell surface of bacteria and yeasts intended to act as whole-cell biocatalysts. Despite their biotechnological potential, protein display technologies remain poorly developed in filamentous fungi. Coupling the lignocellulolytic character of some of them to the cell surface biosynthesis of valuable molecules by a single or a cascade of several displayed enzymes is an appealing target. The development of protein display strategies for filamentous fungi could be based on the field advances in yeasts; however, the unique composition, structure and biology of filamentous fungi cell walls make necessary the customization of the approach to those microorganisms. We will show our progress in generating a well curated and characterized set of *Neurospora crassa* cell wall resident proteins that could be used as molecular anchors. This molecular anchors collection is aimed to allow the control of the abundance, spacing and local environment of the displayed enzymes, critical factors that influence the performance of display-based whole-cell biocatalysts. This work is being supported by SENER-CONACYT 245750 grant.

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# A cytoplasmic Slo3 fragment regulates both Slo3 and Slo1 potassium channels in mouse

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Abstract

Slo3, a pH-sensitive potassium (K+) channel, is closely related to the large-conductance Ca2+-activated channel Slo1. Slo3 channel has a vital role in the membrane hyperpolarization that occurs during sperm capacitation and is essential for male fertility in mouse. Expression of Slo3 protein has been regarded as sperm-specific. Nonetheless, in an exploration of expression databases we detected transcript sequences identified with the Slo3 locus in cDNA libraries from a range of mouse tissues. We analyzed the sequence of these transcripts through computational and experimental approaches and identified two novel Slo3 splice variants, both encoding the final 381 amino acids of Slo3. This sequence corresponds to the C-terminus (CT) of the cytosolic domain of Slo3 channel. Products of these variants were screened both at the transcript and protein level in a range of rodent tissues, revealing the expression of a Slo3-CT isoform in the supernatant fraction of testis, brain and kidney, as well as in blood serum.

To test the functional activity of this soluble protein, we co-expressed a Slo3-CT isoform expressed in brain (CV562866) with either Slo1 or Slo3 channels in a heterologous system (Xenopus oocytes) and recorded membrane currents using two-electrode voltage clamp. Coexpression of Slo3-CT significantly reduced Slo3 currents evoked by a depolarizing pulse to +140mV. Slo3 currents were enhanced in the presence of the accessory subunit Sloβ4, which were suppressed when Slo3-CT is co-expressed. In contrast, co-expression of Slo3-CT with Slo1 significantly increased channel currents at +140mV. Our findings thus highlight the importance of spliced C-terminal variants as a regulatory factor for K+ channels modulation, a proposed general mechanism but still barely explored.

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# Molecular characterization of hydrogenase activity from an Antarctic biohydrogen producing strain.

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Biohydrogen is a clean source of energy that produces during its combustion no harmful byproducts, besides being a potential sustainable energy carrier for future applications. The growing interest on the use of $H_2$ as biofuel is searching for energetically and efficient new production processes, where biohydrogen produced by anaerobic bacteria *via* dark fermentation has attracted attention worldwide. State of the art on biohydrogen production indicates that most of the research has been done with mesophilic or thermophilic but psychrophilic bacteria. Our previous work demonstrated that psychrophilic strain G088 (related to *Polaromonas* genus) is able to produce significant amounts of biohydrogen during dark fermentation from different carbon sources (Álvarez-Guzmán, 2016). Nevertheless, the molecular mechanism involved in hydrogen production by G088 strain is unknown. To identify the enzymes involved on hydrogen production, we designed degenerated primers sets to amplify different subunits of [NiFe] or [FeFe] hydrogenases. The resulting amplicons were verified by Sanger-sequencing and identified as large or small subunits of [NiFe] hydrogenases, displaying 98% identity with hydrogenase complex of *Polaromonas naphtalenovorans*. These genes were subclonated into pET22b(+) plasmid vector (Novagen, MS, EU) for heterologous expression of the catalytic subunits in an *E. coli* strain with increased biohydrogen production.

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# Purification of a α-L-arabinofuranosidase from *Colletotrichum lindemuthianum* race 1472

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α-L-arabinofuranosidase (ABF) (EC3.2.1.55) is a debranching enzyme that catalyzes the hydrolysis of arabinose attached through o-glycosidic anchors to arabinogalactans and hemicellulose. ABF plays an essential role in arabinogalactan hydrolysis because enzymes like endo-β-(1-6)-D-galactanase acts only on desarabinosylated substrates. Additionally, ABFs have potential application in biotechnology like the wine industry, clarification of fruit juices and digestion enhancement of animal feedstuffs. Due to the biological and biotechnological importance of ABFs we purified an ABF from *Colletotrichum lindemuthianum,* a common bean pathogen.

*C. lindemuthianum* race 1472 was grown in PD medium during seven days and then, ABF activity was induced with arabinogalactan (Larchwood) for six days. The extracellular medium was concentrated by ultrafiltration and passed through a desalting column (HiTrap Desalting). The enzyme was purified to homogeneity using anionic exchange chromatography (HiTrap Capto DEAE) as the first step followed by exclusion molecular chromatography (HiLoad 16/600 Superdex 75 prep grade) as last step using Äkta pure system.

As the first result, zymogram assay revealed a single band with arabinofuranosidase activity. On the other hand, chromatogram of anionic exchange showed a single peak with ABF activity, collected fractions from this peak were passed through exclusion molecular column and we obtained a single peak with ABF activity. Finally, we corroborate the ABF molecular weight by SDS-PAGE.

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# Implementation of a PCR-based system for generation of mutants in the phytopathogenic fungus *Ustilago maydis*

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*Ustilago maydis* is a biotrophic, basidiomycete fungus that infects maize causing smut disease or ¨huitlacoche¨. This disease is characterized by the emergence of tumors or galls in the infected host tissues, which contain inside of them masses of teliospores of dark appearance. *U. maydis* has been considered an excellent model for the study of many cellular phenomena including signaling, morphogenesis, dimorphism, mating and pathogenicity. In the last 20 years it has been developed protocols for the efficient generation of mutants, very useful organisms that allow detailed investigations about a variety of molecular aspects of this fungus.

We have recently implemented the PCR-based system for gene replacement mutants proposed by Kamper (2004). This method is based in the amplification by PCR of 1000 pb of the promoter and terminator regions of the gene of interest, including two distinct SfiI restriction sites. These flanking regions are then ligated to a fragment harboring the hygromicin phospotransferase gene (*hph*) (selectable marker) flanked with SfiI complementary sites. The ligation product can be amplified as necessary and be used to transform the fungus.

We evaluated this protocol through the deletion of several genes of different sizes and positions within the genome of *U. maydis*. We transformed in each case protoplasts of FB1, FB2 and SG200 strains, obtaining as many transformants in strain FB2, followed by FB1 and then SG200. These transformants were confirmed by PCR. Because of the number of transformants obtained, simplicity and highly directional strategy of this method, we considered it very suitable for the generation of gene replacement mutants in *U. maydis* in our laboratory.

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# Speeding up the enzymatic reaction of triosephosphate isomerase

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Triosephosphate isomerse (TIM) is a glycolytic enzyme (EC 5.3.1.1) that catalyzes the reversible interconversion of dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate. TIM is a very efficient enzyme, aumenting the reaction rate billions of times compared to that occuring in solution. The reaction is so efficient that TIM has been described as the perfect catalyist, since, its reaction is solely limited by the rate of diffusion of the substrate to the active site of the enzyme (1). Triosephosphate isomerase deficiency is a rare metabolic disorder characterized by a marked decrease in activity of TIM and an accumulation of dihydroxyacetone phosphate in erythrocytes of patients. In consequence, patients suffer from chronic haemolytic anemia, cardiomyopathy, severe neurological dysfunction, and, in most cases, death in early childhood (2). Virtual high-throughput screening has been used to identify molecules, among chemical libraries, that could favourably bind to triosephospahte isomerase and to modify its catalityc properties. We were able to detect one compound, depicted as D1 of the XXX family with these properties. Enzymatic assays confirmed that D1, might speed up the rate of catalyisis of triosephosphate isomerase from *Trichomonas vaginalis.* The possible mechanisms for this observation are discussed. These results migth have implications as a probable therapeutic strategy to treat triosephosphate isomerase deficiency.

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# Comparative analysis of the transcriptome of *Aspergillus caesiellus* grown in pyrene and phenanthrene in hypersaline conditions

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Polycyclic aromatic hydrocarbons (PAHs) are environmental contaminants very harmful to the environment. The use of fungi for PAHs degradation, is considered a useful tool in bioremediation. However, very few of these studies focus on the molecular mechanisms that allow fungi use these compounds as carbon source in halophiles conditions; where they expected to produce functionally stable enzymes to degrade them in hypersaline conditions (2M NaCl). Therefore, deeper knowledge in molecular mechanisms using fungi to degrade PAHs allow bioproducts identification (eg. proteins, biosurfactants) with potential applications in the industrial wastewater treatment. The aim of this project is to analyze comparatively the transcriptome of the halophiles fungus *Aspergillus caesiellus* grown in pyrene and fenentreno in hypersaline conditions (2M NaCl). To accomplish this, the fungus will be expose to a mixture of pyrene and phenanthrene (1:1) as unique carbon source in the presence of 2M NaCl, then, the mycelium will be collect betwen 3 and 36 hours of exposure and will be extract and sequence the total RNA by RNA seq. Later, will be analyze differentially the genes involved in the degradation of pyrene: phenanthrene in hypersaline conditions. This project will provide new information about the molecular mechanisms induced by PAHs in halophiles conditions and possible biotechnological applications derived from *A. caesiellus.*

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# Preliminary study of thermoplastic starch biofilms with additives to inhibit the growth of *Salmonella and Escherichia coli* in blackberry

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**INTRODUCTION.** In recent years, it has become more potential application of less invasive technologies for the environment, for food is not exception. Nowadays, one of our challenges is that perishable foods reach the consumer with the best possible quality, which implies greater quantities of pesticides or chemical preservatives that could damage the health of consumers. One of these new technologies has enabled some foods are coated with thermoplastic biofilms obtained from natural products, which could replace the use of synthetic additives to prolong its shelf life. One of the polysaccharides used is starch, it is a polymeric carbohydrate consisting of a large number of glucose units joined by glycosidic bonds. The plasticizer used is glycerol, for their accessibility and low cost, but this is not enough, it was observed that the biofilm itself does not provide antimicrobial properties, for which the use of essential oils with antimicrobial activity is implemented.

**METHODOLOGY.** The extraction of essential oil of cinnamon was carried out using the technique of stripping steam and was subsequently made a liquid-liquid extraction to obtain a oleoresin. The thermoplastic biofilm was prepared by adding different concentrations of essential oil (0%, 5%, 10%, 20%, 50%). Selective and differential medium for *Salmonella and Escherichia coli* were prepared in Petri dishes with agar MacConkey and *Salmonella- Shigella*. Subsequently, they were inoculated with *Salmonella and Escherichia coli* and little pieces of the thermoplastic biofilm were placed on the middle of the dish.

**RESULTS**. To separate essential oils from cinnamon extraction by steam stripping were used. Ground cinnamon were moistened with distilled water for that the molecules of water vapor to enclose the essential oil, this process was not enough to remove water from the essential oil, so it was necessary to use the technique of liquid-liquid separation in which dichloromethane was added to obtain the final oleoresin from cinnamon. Starch Biofilms were made with different concentrations of essential oil of cinnamon 0%, 5%, 10%, 20% and 50%, and placed on Petri dishes with *Escherichia coli* and *Salmonella*, we observed halos of inhibition in concentrations of 5%, 10%, 20% and 50%, in the control where no essential oil of cinnamon was added, there was no growth inhibition in both bacteria.

**CONCLUSIONES.** It was possible to obtain the essential oil of cinnamon with the technique of stripping steam and liquid-liquid extraction. At low concentrations of essential oil of cinnamon (5%) the growth of *Escherichia coli* and *Salmonella* were inhibited. **ACKNOWLEDGEMENT**. This Project was supported by Programa para el Desarrollo Profesional Docente (PRODEP) No. UPMOR-PTC- 043.

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# Synthesis of catechol melanin from glycerol employing engineered *Escherichia coli*

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Abstract

Background: Melanins comprise a chemically-diverse group of polymeric pigments whose function is related to protection against physical and chemical stress factors. These polymers have current and potential applications in the chemical, medical, electronics and materials industries. The biotechnological production of melanins offers the possibility of obtaining these pigments in pure form and relatively low cost. In this work, *Escherichia coli* strains were engineered to evaluate the production of melanin from supplemented catechol or glycerol.

Results: It was determined that an improved mutant version of the tyrosinase from *Rhizobium etli* (MutmelA), could employ catechol as a substrate to generate melanin. Strain *E. coli* W3110 expressing Mut*melA* was grown in bioreactor batch cultures with catechol supplemented in the medium. Under these conditions, 0.6 g/L of catechol melanin were produced. A strain with the capacity to synthesize catechol melanin from a simple carbon source was generated by integrating the gene Mut*melA* into the chromosome of *E. coli* W3110 trpD9923, that has been modified to produce catechol by the expression of genes encoding a feedback inhibition resistant version of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase, transketolase and anthranilate 1,2-dioxygenase from *Pseudomonas aeruginosa* PAO1. In batch cultures with this strain employing complex medium with 40 g/L glycerol as a carbon source, 1.2 g/L of catechol melanin were produced. The melanin was analysed by employing Fourier transform infrared spectroscopy, revealing the expected characteristics for a catechol-derived polymer.

Conclusions: This constitutes the first report of an engineered *E. coli* strain and a process for producing catechol melanin from a simple carbon source at gram level, opening the possibility of generating a large quantity of this polymer for its detailed characterization and the development of novel applications.

Keywords: Metabolic engineering; melanin; catechol; tyrosinase; *Escherichia coli*.

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# Plant volatiles act in the direct resistance to pathogenic fungi

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Plants respond to herbivory or infection by pathogens with the emission of a complex blend of volatile organic compounds (VOCs). The functions of these VOCs range from direct repellence to signalling effects that induce resistance-related responses in systemic parts of the locally attacked plant, in neighbouring plants, or in even beneficial insects. Here, we focus on the functions that VOCs play in the resistance of plants to fungal pathogens. Cultivars of common bean (*Phaseolus vulgaris*) that exhibit phenotypic resistance to anthracnosis caused by the fungus, *Colletotrichum lindemuthianum*, emitted quantitatively and qualitatively more VOCs than susceptible cultivars. We screened ca. 50 plant-derived VOCs for their capacity to inhibit spore germination and mycelial growth of *C. lindemuthianum* and found that citral, eugenol, terpineol, nonanal and *trans*-2-decanal inhibited mycelial growth. Exposure to VOCs such as citral and eugenol inhibited the mycelial growth in the first minutes. Confocal microscopy revealed an inhibition of apical growth in the hyphae of fungi exposed to citral and eugenol. This inhibition was found to be irreversible, because the fungus did not recover growth after removal of the VOCs from the atmosphere. We conclude that VOCs have potential to be used as natural, organic fungicides in sustainable agriculture.

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# Effect of polyphenols from the leaves of Mexican avocado (Persea americana var. drymifolia) over the gene expression of Methicillin-Resistant *Staphylococcus aureus*

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The constant use of antimicrobials, causes an increase in the number of multiresistant pathogens such S. aureus. This is a pathogen that adapts daily new generations of antibiotics. A new strategy to combat pathogens, is the use of plant secondary metabolites, which has the important biological activity, be bactericides. In the southern part of Nuevo Leon criollo avocado leaves as ethnobotanical used remedy against infections. The aim of this work is to determine the bactericidal potential of ethanol extracts of total polyphenols of avocado leaves, and observe the change in gene expression of methicillin resistant Staphylococcus aureus (MRSA). Here we evaluated the total polyphenols extracted for leaves of Mexican avocados from the cultivar Maria Elena against the MRSA strain µ3 . It was determined the MIC between the concentration of 100- 200µg of polyphenols. For the WBC was observed at a concentration of 200µg of polyphenol avocado leaves.

To determinate the mechanism of the polyphenols on the bacteria, the µ3 strain of S. aureus (Methicillin resistant and vancomycin intermediate) was stressed with the polyphenols by lapse of one hour, whereupon the total RNA was extracted and transformed into cDNA. The total gene expression was hibridized in a specific microarray. The data was analyzed with the statistic Z score, comparing the results of the control against the experimental condition were observed genes that scored z +/-2 were investigated and correlated according to their function in the bacterial system.

It was observed a change in gene expression of S.aureus MRSA, manage for the polyphenols, we observed a series of down regulated genes involved in bacterial communication, which corresponds the *agr* operon and the gene involved in *RNA III* activating virulence factors. Furthermore the *rpl* operon and *inf*, are essential for protein synthesis. For genes over expressed, is *CHB* gene involved in biofilm formation, ACPD; in the metabolism of the cell membrane and Clp operon; which it is responsible for the production of proteolytic proteins, which if not regulated by the bacteria, causing lysis thereof. The analysis of the extracts with bioactive compounds against bacterial strains resistant to drugs represents an opportunity to find new antibiotics in nature.

In this paper, we show that native cultivars of southern Nuevo Leon avocado (*Persea americana* var. *Drymifolia*), have an important effect in vitro against a bacterium of great importance with a bactericidal effect. We also found that the extract significantly suppressed many important genes expression in many important process like Quorum sensing, virulence, protein synthesis, cell wall etc. This gives us the opportunity to search for specific compounds that inhibit the growth and development of the bacteria.

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# Effect of mitochondrial inhibitors on the viability of cancer stem-like cells of breast cancer MCF-7

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Recurrence and metastasis of breast cancer (BC) is the first cause of death in patients with this disease. Recently a small subpopulation responsible for cancer recurrence and metastasis has been identified and called cancer stem cells (CSC)[1]. A common cause of treatment failure in multiple malignancies is resistance to conventional chemotherapy (by example, doxorubicin and taxol in BC treatment) and radiotherapy, common features of CSC. In addition conventional therapies are not selective against CSC causing poor prognosis and death risk in cancer patients. Therefore, it is mandatory the search of novel therapeutic strategies targeting CSC to treating malignant progression.

Reprogramming of energy metabolism is a hallmark of cancer cells that can be used as a possible therapeutic target. Previous work in our lab determined that oxidative phosphorylation is the energy pathway that supplies the most amount of ATP (87% vs. glycolysys 13%) in breast cancer stem cells of MCF-7[2]. The above indicates the need of evaluate an antimitochondrial therapy for targeting breast CSC.

In this work we enriched CSC fraction in monolayer culture of MCF-7 by expose cells to combinations of microenvironmental stress conditions (hypoxia, hypoglycemia and antineoplastic drugs). Breast CSC like phenotype (BCSC) was verified by stemness markers CD44+, CD24-, ALDH+ and Oct 3/4 (wich increased 4-20 fold), the expression of P-glycoprotein (with an overexpression of 6 fold) and their invasiveness (>80%) vs MCF-7.

Subsequently, mitochondrial (Oligomycin, Casiopeina IIgly, α-Tea and α-Tos), glycolytic (gossypol, 2-deoxyglucose, 3-bromopyruvate and Iodoacetate) inhibitors, Canonical (doxorrubicin, paclitaxel, cisplatin, 5-fluorouracil) and non canonical drugs (celecoxib and sulindac) were evaluated at different concentrations during 24 hours in a 96 well plates to determine their effect on viability in BCSC. We obtained that Casiopeina IIgly, α-Tea celecoxib and sulindac were more potent at low concentrations (IC50 from 0.8 to 30 µM) compared to glycolytic inhibitors (IC50> 100 µM). These data demonstrate that antimitochondrial therapy, at low concentration, reduce the viability of BCSC.

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# Meniscal Cell Membrane Express Ectopic IF1-ATP Synthase and Its Expression is Regulated by TNF-α and IL-1β.

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The meniscus is a semicircular structure fibrocartilage tissue that is located between the articular surface of the femur and tibia at the knee . The outer region is a vascularized and innervated area, while the inner region is avascular and is not innervated . The matrix contains 75% collagen type I and the rest of proteoglycans , adhesion proteins and elastin. Collagenous fibers are arranged in a circumferential pattern that gives strength and stamina to absorb the shock and load transmission during movement of the joint and limitation of flexion and extension. The partial or complete removal of the meniscus leads to deformation of the joint, increases the contact force of articular cartilage and therefore the development of osteoarthritis. Joint injury, as well as degenerative joint diseases are characterized by increased activity of the pro-inflammatory cytokines as interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α). This upregulation of pro-inflammatory mediators in injured or arthritic joints, when combined with cytokines produced endogenously by meniscal cells, may act to suppress matrix biosynthesis, increase enzymatic degradation and vascularization. The influence of these factors on repair capacity of the meniscus is not complete known. The goal of this study was to investigate the effect of pro-inflammatory cytokines as TNF-α and IL-1β on expression of ectopic inhibitor protein (IF1) of ATP synthase on membrane cell of human meniscus and its localization on healthy and injury meniscus. The goal of this study was to investigate the effects of the inflammatory cytokines IL-1β and TNF-α on the expression of ectopic IF1 in meniscal cell membrane as well as healthy and injured meniscus.

Human meniscus cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 2.5% heat-inactivated fetal bovine serum for different hours in the presence of IL-1β and TNF-α. The cellular responses were analyzed by fluorescence confocal microscopy, flow cytometry and immunohistochemistry using a specific anti-IF1 antibody (Abcam) and Cy3 and FITC secondary antibodies (Invitrogene). To differentiate mitochondrial from membrane IF1 the cells were cultivated in presence of absence of Triton-X100. Our results show the subunit IF1 of ATP synthase were detected on the plasma membrane of meniscal cells as well as on healthy and injured meniscus. The expression changes depending the area (superficial or inner) and type of meniscus injured. The expression of IF1 increased with the exposition time with both IL-1β and TNF-α. The major expression was at 12 hr and decreased after this time. There is a better response en presence of TNF-α than IL-1β**.** In conclusión, we show a new ectopic localization of IF1 of ATP synthase on meniscus cell membrane. This IF1 is up-regulated by inflammatory cytokines TNF-α and IL-1β. This distribution could be related to angiogenesis produced during OA and perhaps ATP production for proliferation and normal function of the chondrocyte . Previously it has been reported that other mitochondrial complexes are affected in OA. Therefore IF1 can be a target in studying metabolism and vascularization of the meniscus and cartilage in OA development.

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# Establishment of an obesity model to evaluate hepatic steatosis

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Área: 6. Medicina, Salud y Nutrición.

Sedentary lifestyles and poor dietary choices are contributing to a weight gain epidemic in westernized societies. Recent epidemiological studies suggest an increased risk of cardiovascular disease (CVD) and type Ⅱ diabetes in overweight and obese individuals. The incidence of the metabolic syndrome and nonalcoholic fatty liver disease (NAFLD), which can precede the development of CVD and type Ⅱ diabetes, are also increasing. Nonalcoholic fatty liver disease (NAFLD) is a disease comprising a continuum of liver damage ranging from simple steatosis to nonalcoholic stetatohepatitis (NASH) and cirrhosis. Hepatic steatosis refers to an excess accumulation of lipids, primarily triglycerides (TG), which is the hallmark of NAFLD. General overnutrition, particularly when being rich in fat and/or sugars like fructose, is being discussed to be key factors in the development of NAFLD. However, molecular and pathological changes involved in the onset and the progression of NAFLD are not yet fully understood and therapeutic options are still rather limited. Therefore, a better understanding of the alterations causally involved in the early stages of NAFLD is desirable to improve prevention and intervention strategies. Animal models of NAFLD give crucial information, not only in elucidating the pathogenesis of NAFLD but also in examining therapeutic effects of various agents. Thus in this work we used male New Zealand rabbits fed with a high fructose, high fat and high cholesterol diet (HFFCD) in order to produce NAFLD. The HFFCD group was fed a chow diet supplemented with 30 % fructose, 10 % animal fat and 5% cholesterol because HFFCD diet can induce more prominent alterations than a regular chow diet. We evaluated serum levels of hepatic enzymes, aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALKP), gamma-glutamyl transpeptidase (□-GT) and lipid profiles (cholesterol, triglycerides, HDL-cholesterol) at the beginning of the experiment and at 7 and 12 weeks of HFFCD. We observed an elevation in the hepatic enzymes, especially in ALT (36.7% ± 4.8) as well as in the triglycerides (40.7% ±7.6) levels, suggesting this HFFCD diet is altering some hepatic functions which would allow us to establish a rabbit model of hepatic steatosis to be further evaluated with more parameters.

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# 2-methoxyestradiol and dichloroacetate effects on A-549 and MRC5 cells grown under hypoxic conditions.

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**Background**: Fibrotic tissues and solid tumors contain hypoxic regions that are involved in disease progression. Cells growing under this condition are known to be more resistant to currently available treatments than normoxic cells. In this regard, dichloroacetate (DCA) activates pyruvate deshydrogenase and has been used to avoid lactic acidosis in cancer, while 2-methoxyestradiol (2ME) has been used to stop neoplastic progression because of its pro-apoptotic, anti-angiogenic and anti-proliferative proprieties. **Objective**: The aim of the present study was to analyze DCA and 2ME effects on A549 (human adenocarcinoma cells) and MRC5 (human fibroblasts) cell proliferation under normoxic and hypoxic conditions. **Methods**: Cells were cultured in normoxic and hypoxic conditions during 72h with DCA alone or in combination with 2ME at different concentrations. Cell growth was determined by N-hexa-methylpararosaniline staining assays. Cell growth comparisons among different culture conditions were analyzed by the Mann–Whitney “U” test. **Results**: There was a DCA or 2ME dose-dependent inhibitory effect on cell growth in both cell lines cultured in normoxic conditions. 2ME had a minor inhibitory effect on cell growth compared with DCA in A549 and MRC5 cells in hypoxia. The combination of both drugs (2ME 10µm and DCA 40mM) decreased A549 cell proliferation in normoxia with no effect in hypoxia. In contrast, both drugs induced a decrease in MRC5 growth rate in hypoxia with little effect in normoxia. **Conclusion:** Our results suggest that the tested drugs’ effect on cell proliferation was different in epithelial and mesenchymal cells under hypoxic conditions. The combine use of these drugs could improve fibrosis treatment.

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# Analysis of the expression of miR-15a, miR-16-1 and miR-193a in circulation and its correlation with the expression of WT1 in patients with nephrotic síndrome

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**Introduction**. Nephrotic Syndrome (NS) is a kidney condition characterized by massive loss of protein in urine, associated with hypoalbuminemia, hyperlipidemia, hypercoagulable and edema. Kidney disease is a public health problem in Mexico, with high costs for clinical diagnosis and treatment. Patients with nephrotic syndrome Cortico Resistant (SNCR) do not respond to treatment with corticosteroids and these have a poor prognosis due to the decrease of basal expression of tumor gene Wilms 1 (WT1) in podocytes and / or mutations in this gene or in some others like Nephrin and Podocalxin, responsible for podocyte physiology and stability. Among the mechanisms of regulation of expression of the WT1 gene epigenetic modulation is included, it has recently been reported that some microRNAs downregulate WT1 therefore the aim of this study was to determine the correlation between expression of WT1 and levels miR-15a of, miR-16-1 and miR-193a in serum of patients with SN.

**Materials and methods**. the relative expression of WT1 and its nuclear localization or cytoplasmic in 10 patients with SN biopsies by qPCR and immunofluorescence respectively, and the relative expression of microRNAs 15a, 193a 16-1 and serum was analyzed in 21 patients with NS and 10 controls without renal disease by qPCR ($2^{-\Delta\Delta CT}$).

**Results.** In the study we found a decrease in mRNA and protein WT1 in podocytes in renal biopsies and urinary sediment of patients with nephrotic syndrome Corticosensible (SNCS) and SNCR, presenting a primarily cytoplasmic localization in both groups. In the relative expression of miR-15a and miR-16-1 was found a decrease in patients with SN, showing significant difference in miR-16-1 NS patients compared to healthy subjects ($p = 0.019$), while the miR-193 expression was homogeneous between groups.

**Conclusions.** Our results suggest a decreased basal WT1 expression and a predominant cytoplasmic localization, which is associated with the loss of its activity as a transcription factor in patients with SNCR. Finally, WT1 expression showed no correlation with the microRNAs analyzed in sera from patients with SN.

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# Purification of human Paraoxonsae 1 (PON1h) from blood inhibits biofilm formation on *Aeromonas caviae* Sch3

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Human serum paraoxonase 1 (PON1h; EC 3.1.8.1) is a glycosylated protein with an estimated molecular mass of 43 kDa, consists of 354 amino acids and it is a calcium-dependent esterase that hydrolyzes aromatic carboxylic acid esters, toxic organophosphate compounds, and lactones. The physiological function is still unclear. The enzyme is primarily synthesized in the liver and is secreted into the blood, where it is associated exclusively with high-density lipoproteins (HDLs) this makes the purification is a problematic issue and although there is several protocols of purification most of them consists of multi-steps and longer times of purification. By having an access to purified PON1h it would afford several advantages such as facilitate its functional characterization and to know more about biologically relevant activities or applications on medicine in the complete absence of other serum proteins. In this work PON1h was purified with a single step procedure.
Human serum PON1 was purified by an AKTA-Fast Performance Liquid Chromatography (FPLC) system using a HiPrep Phenyl FF (high sub) 10/16 column for hydrophobic interaction chromatography. Quorum sensing is mediated by the synthesis, sensing and uptake of small molecules chemical signals which are known as autoinducers like N-acyl homoserine lactones. When these autoinducers attain a critical threshold concentration, they trigger various genes, commonly associated with antibiotic resistance, release of virulent factors and biofilm formation. Bacteria residing within biofilms are found to be 1000 fold more resistant to antibiotics and are essentially insensitive to the host immune response. It has been observed that biofilm formation is associated with many infectious diseases and is a major concern for immunocompromised patients. *Aeromonas caviae* Sch3 is a Gram-negative bacterium that lives in aquatic habitats. It has the ability of biofilm formation. The PON1h purified significantly inhibited the biofilm formation determined by the crystal violet assay and also observed by Scanning Electron Microscopy. In conclusion, the present study illustrates that PONh1 can be purified with a single hydrophobic interaction chromatography and it is active inhibiting biofilm formation in *Aeromonas caviae* Sch3. The pure enzyme might be used in some way to prevent biofilm formation.

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# Protein levels of IRE1, ATF6 and PERK (Unfolded Protein Response mediators) in alkali corneal lesion in rat

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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, these are IRE1, ATF6 and PERK. 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 corneal lesions with alkali, in the way to see the sights in human corneal diseases like keratocone.

Alkali corneal lesions was provoked in Wistar rats with NaOH 1M. The corneal tissue was examined at 0, 24, 48 and 72 h after lesion. The corneal damage and tissue recovery was determined by fluorescein and Hematoxylin-Eosin staining. The protein levels of UPR regulators (IRE1, ATF6 and PERK) was measured by immunohistochemistry 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. Corneal lesion induced inflammatory cells infiltration and loss of collagen fibers arrange in the stroma, at 24 h. When we explored the protein levels of the UPR pathway regulators, we observed an *in situ* increment of IRE1 levels (at 48 h) and ATF6 levels (at 48 and 72 h) induced by NaOH lesion. On the other hand, the corneal PERK levels were not modified by alkali injure. These results suggest that UPR pathway could be activated in corneal lesions and this cellular response might be associated to the corneal damage process.

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# Effects of *Spirulina* maxima on physical performance and antioxidant status in athletes

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**Introduction:** Among the objectives of an athlete is improving his physical performance that is favored by the optimum utilization of oxygen during aerobic metabolism, under this condition reactive oxygen species (ROS) are generated, which, modify the antioxidants/ROS athlete ratio. But if his diet does not contain enough antioxidants or precursors thereof, ROS could increase significantly leading the athlete to an state of oxidative stress.

Furthermore, the cyanobacteria *Spirulina maxima* has different biological activities and antioxidant activity is included (mainly attributed to phycocyanin pigment).

The aim of this study was to determine if *Spirulina maxima* improves physical performance and the antioxidant system in high performance male swimmers, when it's administered as a dietary supplement at a dose of 6 g per day.

**Design and Methodology:** This is a pilot study, single blind, placebo-controlled, study which involved 14 male swimmers of the representative team of the National Autonomous University of Mexico, whose ages were between 9 and 28 years old. Randomization of groups (*Spirulina* and placebo) was performed by stratifiyng by age, so accomplished to date baseline. Each Athlete was carried out biochemical, morpho-functional and sports tests before (baseline) and after treatment (post). The treatment was carried out for 12 weeks and involves taking 12 pills daily (*Spirulina* or placebo) divided into three doses per day. During treatment, athletes attended his training and took a diet according to his weight and body composition. Compliance with treatment and diet, as well as events that occurred were monitored through weekly surveys and handover of packaging of pills.

Tests for maximal oxygen uptake (VO2max), critical swimming speed, and speed swimming 300m (T300), lactate threshold in progressive tests 300m, antioxidant enzymes, and blood lipids, among others, were obtained before and post-treatment.

**Results:** With the data obtained at before and post-treatment tests, delta values and delta percentages for each variable studied were calculated and analyzed statistically using the Graph Pad Prism version 5.0, considering an $\alpha = 0.05$ for all tests.

As expected, some variables (age, proportion of nutrients in the diet) were not different between both groups,. However, significant differences were observed between before and post-treatment in Spirulina group: % delta T300 ($p = 0.05$), % delta concentration of reduced glutathione (GSH) ($p = 0.0012$ difference between means 82.09 ± 21.5 μg/mL), delta $VO_2$max ($p=0.0256$ difference between means 4.45 ± 1.9 mL /kg / min). An F-test comparison of variance was performed finding significant difference in % delta lactate AUC ($p = 0.0132$).

**Conclusions:** A trend toward improvement in aerobic performance of athletes who consumed *Spirulina* as well as a tendency to improve antioxidant status is observed.

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# Preliminary study of *Coriandrum sativum* seed consumption on dysglycemia and dyslipidemia models.

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**Introduction:** The chronic and degenerative diseases are a severe health problem, occupying the first place of worldwide mortality. The insulin resistance, metabolic syndrome and type 2 diabetes mellitus are some of them, and these are caused by hypercaloric diets consumption. It's widely described that the hypercaloric diets are involved in tissues damage by the dysglycemia and dyslipidemia characteristics in these pathology. The hypertriglyceridemia associated with HDL decrement are considered dyslipidemic status, as well as, hyperglycemia in fasting also is part of metabolic disorder. The classical pharmacology treatments are based on metformin or pravastatin consumption; however, Mexican traditional medicine has proposed to *Coriandrum sativum* (coriander seed) as an alternative of the therapeutic approach. **Objective**: Study the effect of *Coriandrum sativum* seed in Long-Evans rats with dysglycemia and dyslipidemia. **Methodology:** After 2-months of 5008 LabDiet diet consumption, rats develop fasting hyperglycemia and dyslipidemia (hypertriglyceridemia and HDL decrement). Groups of 5 rats were separated in independents cages, and administered with 5008 diet (L), 5008 diet mixed with seed (L+S), 5008 diet + glucose (25%) in water (L+G), 5008 diet mixed with seed + glucose (25%) in water (L+S+G) and 2018 diet as control was used since the begin (normocaloric diet). Diets were administered for 3-months more. At the end of 3-months, insulin, glucose, triglycerides and HDL were quantified by ELISA and spectrophotometry methods. Additionally, zoometric indexes were measures: weight, height, abdominal circumference and body fat percentage. **Results:** The mean weight of control group 2018 LabDiet fed was of 422.3 ± 10.3 g, with a height of 25.5 ± 0.2 cm, meanwhile L, L+S, L+S+G groups don't show differences, however, L+G group increases in 25% of body weight. The abdominal circumference and body mass index were increased in L and L+G groups (9.2%, 4.6% and 20.7%, 21.5; respectively). The body fat percentage was increased in all groups (L = 2.4%; L+S = 1.5%; L+G = 8.2% and L+S+G = 2.0%), being the rats fed with seed least affected. The fasting glucose and triglycerides showed difference only in L+G group (26.6% and 60.1%). The HDL concentration was dramatically diminished in L (14.5%), L+G (31.6%) and 22.9%). Finally, the insulin levels were elevated markedly in L (19.3%), L+S (15.7%), L+G (39.2%) and L+S+G (28.8%). **Conclusion:** Our results showed that *Coriandrum sativum* seed consumption is able of regulate some effects of hypercaloric feeding as weight, body mass index, abdominal circumference, even glucose and triglycerides serum, however, don't show improved on HDL and insulin.

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# Coxpression of progesterone receptors and TGF-β in Ovarian epithelial cancer

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## Abstract

Ovarian epithelial cancer derive from the surface epithelium of the ovary is the most common ovarian neoplasms and the more lethal [1] due to it is detected in advanced stages of the disease and that 60-70% women presents recurrence and becomes chemoresistant [2]. These carcinomas are classified according to their molecular, genetic and histopathologic features; the tumors serous are the most frequent and account for about 75% of cases [3, 4].
Steroid sex hormones have been associated with the development of ovarian cancer. However, has been observed that exposure to progesterone is a protective factor for this malignancy, whereas the presence of its receptor (PR) has been associated with a good prognosis for serous and endometrioid subtypes [6, 7]. On the other hand, is known that the beta transforming growth factor (TGF-β) a protein whom functions are inhibit proliferation of ovarian surface epithelium in ovulation [8], which could be regulating anti tumor effects of progesterone. Therefore, TGF-β and progesterone could be acting synergistically and induce apoptosis in ovarian epithelial cells [9]. But mutations in TGF-β and its receptors RI and RII have been related with the induction of several subtypes of epithelial ovarian cancer by increasing its expression up to 75% in serous and clear cells [10, 11]. The aim of this study was evaluate the coexpression of PR and TGF-βRII in ovarian carcinomas and the association with tumor cell proliferation.
The results shows that 53.8% of serous high-grade carcinomas were positive for PR, while TGF-βRII was expressed a 23%, the coexpression with 7.6%. In addition, in the case of borderline tumors, these exhibited a 76.9% for PR and 46.1% for TGF-βRII and with a coexpression of 46.1%.
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# Effect of *Moringa oleifera extract* on glutaminase-1 activity in a breast cancer murine model

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*Moringa oleifera* is a plant that has been reported to present antitumor activity. Furthermore, hydroalcoholic extract of *Moringa oleifera* leaves decreases heme oxigenase-1 activity and preserves mitochondrial respiratory complexes in a diabetes mellitus type 1 murine model. Therefore, it is possible that its compounds interact with mitochondrial enzymes. However, the mechanism involved in antitumor activity of this plant in breast cancer has been poorly studied. On the other hand, cancer cells proliferation demand increased biomass production and energy. Catabolism of L-glutamine provides macromolecules through recycling Krebs cycle intermediates. Glutaminase regulates glutaminolysis pathway, in cancer, isoform 1 (GLS-1) is overexpressed. Hence, the general objective of this study was to determine the effect of hydroalcoholic extract of *Moringa oleifera* leaves over GLS-1 activity in a murine breast cancer model. Female rats Sprague-Dawley were used and divided into control, untreated breast cancer induced with N-Nitroso-N-ethylurea (ENU), and cancer treated with extract (500 mg/kg body weight) during 1 month. The effect of the extract on glutaminase-1 was evaluated in mitochondria by measuring enzymatic activity through a spectrophotometric assay. Results showed that in extract-treated breast cancer group, GLS-1 activity was different compared with the untreated group. Furthermore, docking studies realized in the catalytic site of glutaminase-1, of compounds present in leaves extract, showed that polyphenols with better binding energy were quercetin (-14.4171 kcal / mol), catechin (-14.4128 kcal / mol) and rhamnetin (-14.3151 kcal / mol). Thus, our results suggest that compounds of *Moringa oleifera* leaves extract possibly interact with important residues in GLS-1 and regulate its activity.

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# Antagonistic Activity of a *Lactobacillus* sp Strain Isolated from Environment against Bacteria Associated with Foodborne Diseases

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**Abstract.** Foodborne diseases are a worldwide health problem, due among other factors to development of resistance to many drugs that show most of the causative agents, including bacteria; constituting an alarming situation. The potential use of probiotic bacteria as *Lactobacillus* represents a promising alternative, being that it may exert a protective effect through various mechanisms such as: progressive pH change of the habitat where they develop, competition for nutrients and secondary metabolites production with antagonistic activity (lactic acid, propionic acid, acetic acid, hydrogen peroxide, ethanol), low molecular weight peptides (bacteriocins), among other compounds. The aim of this study was to evaluate the antagonistic effect of a *Lactobacillus* sp strain isolated from environment against bacteria associated with foodborne diseases. *Methodology*. From an environmental sample it was isolated and identified, by conventional techniques of microbiology and molecular biology, a strain belonging to the group of lactic acid bacteria. The antimicrobial activity of the isolated strain was tested by antagonism essays through agar well diffusion method and double layer method against various bacteria associated with foodborne diseases: *E. coli* ATCC 25922, *E. coli* O157: H7 ATCC 70092, *S. typhi* ATCC 14028, *B. abortus* S19, *B. abortus* RB51, *V. cholerae* 01, *V. cholerae* 01 (Inaba), *V. cholerae* 01 (Ogawa), *V. parahaemolyticus* ATCC 17802, *S. aureus* ATCC 25923 and *L. monocytogenes. Results*. By amplification and r16S gene sequencing was identified that the strain belongs *Lactobacillus* sp genus. The antagonism essays by both techniques showed a wide range of inhibition against all strains tested, showing a greater effect on the double layer test, suggesting that the bacteria produces a greater inhibition in liquid media than in solid media. *Conclusions*: The *Lactobacillus* sp strain isolated from environment presents a wide antagonistic capacity against strains associated foodborne diseases, offering a potential probiotic alternative.

**Keywords:** *Foodborne diseases, Lactobacillus sp, Antimicrobial Activity, Probiotic.*

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# Hypoxia modulates the expression of the cytochrome P450 isoforms CYP1A1, CYP2S1, CYP3A7 and CYP24A1 in cancer

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**Background:** Low levels of oxygen (hypoxia) and necrosis have been reported in solid tumors. The hypoxic microenvironment induces increases in glycolytic activity and vascularization, among other physiological changes, but in cancer this changes could lead to a more aggressive behavior. In fact, hypoxia could contribute to anticancer drug resistance as it is affecting drug metabolism due to low levels of oxygen. But hypoxia could modulate the expression of certain profiles of genes which define the presence or absence of drug metabolizing enzymes as some cytochromes P450 isoforms. Our aim was to define which isoforms could be affected by hypoxia using an expression microarray analysis in an in vitro model of cancer.
**Methods:** We examined profiles by means of whole genome expression microarrays (Affymetrix U133 Plus 2.0 Array) on hepatoblastoma cell lines Hep3B and HepG2 in conditions of hypoxia compared to normoxia from free repository databases (ArrayExpress and Gene Expression Omnibus). Microarray analysis was achieved by means of CEL files of the Partek Genomics Suite 6.6v software (Partek Incorporated, Saint Louis, MO). Probe sets were summarized by means of Median Polish and normalized by quantiles with no probe sets excluded from analysis. Background noise correction was achieved by means Robust Multi-chip Average (RMA) and data were log2 transformed. Data grouping and categorization was achieved by principal component analysis (PCA). Hierarchical clustering was based on the dissimilarity of samples (Euclidian method) by means of average linkage. We selected the cytochrome isoforms genes with a fold change $\leq$ 1.2 (ANOVA; FDR<0.05) and the results were confirmed by RT-PCR.
**Results:** We found 4 candidates genes in hypoxic samples of liver cell lines that are modulated in cancer. First, the expression of the cytochromes P450 isoforms CYP1A1 and CYP3A7 were down-regulated in hypoxia (-3.37 and -3.25 fold change, respectively) using Hep3B cell line. Also CYP2S1 and CYP24A1 were up-regulated with al least twice fold change compare with normoxia in HepG2 cell line. In the case of CYPS1, the increase of the expression is progressive, with 1.28, 2.14 and 2.9 fold change at 4, 8 and 12 h of hypoxia. The isoform CYP24A1 also shown a gradual escalation with 1.17, 1.81 and 2.12 fold change at 4, 8 and 12 h of hypoxia, respectively.
**Conclusions:** Hypoxia modulates CYP1A1, CYP2S1, CYP3A7 and CYP24A1 cytochrome P450 isoforms, which might compromise drug effectiveness in cancer treatment.

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# MicroRNAs profiling in breast cancer stem cells-like ($CD44^+/CD24^-$) identify members of WNT/β-catenin signaling pathway as targets of let-7c-3p

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**Background:** The cancer stem cells (CSC) hypothesis postulates that tumors are maintained by the self-renewing of CSC subpopulation (CD44+). The CSCs are capable of differentiating into non-self-renewing populations that constitute the bulk of tumor. CD44 has function in cell division, migration, adhesion, and signaling. Of clinic interest, CSCs not only are responsible for tumor heterogeneity, but also exhibits a more aggressive phenotype and resistance to chemotherapy, thus they are considered novel therapeutic targets. CSCs depend on expression of OCT4, NANOG, and SOX2 transcription factors which are the master regulators of stemness. These genes maintain the pluripotence and self-renewal of CSCs. MicroRNAs are non-coding small RNAs that repress expression of genes involved in development, cellular proliferation and differentiation. MiRNAs also have been implicated in regulation of CSCs capabilities; thus a better understanding of the role of miRNAs could helps us to the identification of novel promising biomarkers and therapeutic targets in breast cancer.

**Results:** Here we isolated a $CD44^+/CD24^-$ enriched subpopulation from MCF-7 breast cancer cells line using FACS. In order to evaluate the potential contribution of miRNAs in the maintenance of $CD44^+/CD24$ stem cell phenotype, we profiled 667 miRNAs using stem-loop RT-qPCR on TaqMan Low Density Arrays. Our data showed that 41 miRNAs were significantly ($FC>1.5$; $p<0.05$) modulated (40 downregulated and 1 upregulated). Bioinformatic analysis showed that these miRNAs potentially targets genes involved in maintaining the self-renewal and differentiation of MCF-7 *stem cell-like cells* including SOX2, NANOG, OCT3/4 and FOXD3, as well as WNT signaling pathway members (GSK3-β, β-catenin, APC and AXIN2) which carries let-7c-3b binding sites at their 3´UTRs. Here we focused in the study of let-7c-3p, a miRNA downregulated in $CD44^+/CD24^-$ cells with unknown functions in cancer. Restoration of let-7c-3p in MCF-7 cells inhibits GSK3-β suggesting a role in stemness. Targets validation using luciferase assays, in progress; will help us to define the role of let-7C-3p in the maintaining the stem cell-like phenotype and its potential as a target in breast cancer therapy.

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# In the HCC827 lung adenocarcinoma cell line, Erlotinib induces apoptosis associated with the expression of immunogenic cell death markers

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Lung Cancer is the leading cause of cancer-related mortalities worldwide. Several studies have reported that EGFR mutation-positive patients treated with Tyrosine Kinase Inhibitors (TKI) have better efficacy and clinical outcomes than EGFR mutation-positive patients treated with conventional chemotherapy (platinum-based double chemotherapy). Erlotinib, a reversible TKI, induces in sensitive cancer cells, cell cycle arrest and apoptosis, increasing free progression survival of patients, but not overall survival. This aspect is doubt because most patients develop acquired resistance to EGFR-TKI treatment. In this condition, patients are treated using conventional chemotherapy. Some reports indicate that some cytotoxic drugs induce a new modality of cell death called immunogenic cell death (ICD) in which the antitumor immune response is stimulated. During this process, intracellular molecules acquire a membrane distribution or they are released. This relocalization gives to these molecules the potential to activate the immune system. According to the Committee for Cell Death Studying, the ICD require spatial-temporal expression of at least three indispensable molecules known as the hallmarks of immunogenic cell death. An early event is the membrane exposition of calreticulin acting as “eat me” signal. Later, the cytosolic ATP is liberated to the extracellular space and finally the nuclear protein HMGB1 protein is released outside the cell, and they act as “find me” signals. Currently, no information whether tumor cell death caused by TKIs is related to ICD. In the present study we analyzed in the lung adenocarcinoma HCC827 cell line, with *EGFR*-activating mutation, whether cell death caused by Erlotinib is mediated by ICD markers expression. The $IC_{50}$ values of Erlotinib, CDDP and their sequential combination were established after 48 h of culture. Analysis of cell cycle distribution were assessed by flow cytometry. During the 36-48 h of final culture incubation, percentages of apoptotic and necrotic cells induced by these treatments were measured using flow cytometry. Caspase-3 activity from cell lyse was quantified employing fluorometry. Also, intracellular ADP/ATP ratio were quantified by luminometry. HMGB1 from culture supernatants was quantified by ELISA. Previous to the appearing of morphological changes related to apoptosis, calreticulin exposed on cell surface was detected by flow cytometry. To analyze in residual cells whether the treatments alter the HMGB1 intracellular distribution and contribute to increase the HMGB1 release, HMBG1 distribution was observed by immunofluorescence. Our results shows that Erlotinib induced cell cycle arrest in $G_0/G_1$ phase at low concentrations and apoptosis mediated by caspase-3 activation in concentrations above 40 nM. During the apoptotic process, increase of ADP/ATP ratio and non-significant increase of HMGB1 were found. As a different pattern of nuclear localization of HMGB1 was observed in treated compared to non-treated cells, which suggests higher nuclear concentration, this phenomenon might explain the decrease detected in the culture supernatants. Respect to calreticulin, a slightly increment in the membrane-exposed calreticulin was detected. When cells were treated with combination of cytotoxic drugs, similar data were obtained, except for the HMGB1 release and cell distribution. This treatment increased HMGB1 concentration in culture supernatants, event mediated perhaps by nuclear to cytoplasmic shuttling of HMGB1 caused by Cisplatin.

In conclusion, in the HCC827 lung adenocarcinoma cell line the tyrosine kinase inhibitor Erlotinib induces, according to the concentration, cell cycle arrest or apoptosis. Apoptosis was associated with the expression of immunogenic cell death markers. As ICD stimulates an efficient antitumor immune response, studies monitoring changes in the antitumor activity of the distinct sub-populations of immune cells in patients is required. Also, searching of ICD-biomarkers are necessary as they might have relevant clinical and therapeutic implications in lung cancer treatment.

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# Regulation of respiratory chain by *Moringa oleifera* leaves extract in a breast cancer murine model

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Cancer cells require mitochondrial metabolic pathways to maintain its proliferation. However, this kind of cells does not depend of oxidative phosphorylation for ATP synthesis. So, the possible function of the respiratory chain could be associated to the signaling process of the reactive oxygen species to maintain the oncogenic profile. In the search of selective antitumoral compounds targeting mitochondrial enzymes, called “mitocans”, polyphenols could be an alternative owing to their direct effect over respiratory complexes. Previous studies with *Moringa oleifera* extract showed antitumoral activity attributed to the high concentration of natural compounds. Thus, the objective of this study was to determine if the antitumoral effect of the *M. oleifera* leaves extract involves the increase of the reactive oxygen species in a breast cancer murine model. Based in the development of an N-nitroso-N-ethylurea (ENU) breast cancer model, we worked with a control group, an untreated group, and a group treated with a dose of *M. oleifera* extract (500 mg/kg body weight). All rats were sacrificed 3 months after cancer induction, liver, mammary glands, and tumors were collected and processed for the measurement of respiratory activity of the complexes, enzymatic activity, and immunoblot in the different tissues. The respiratory complexes density decreased and the respiratory activity was modified possibly as a result of the extract. Also the production of ROS was modified in the Moringa treated group, which is consistent with the reported in studies with cell cultures.

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# (-)-Epicatechin flavonoid treatment improves the mechanical heart performance in healthy mice

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ABSTRACT

Our previous results showed that Epi treatment induced increase in the cardiac mass, causing a symmetrical growth of the left ventricular free wall and the septum. Remarkably, these cardiac changes went together without any sign of fibrosis. This pattern has been associated with ventricular enlargement athlete's heart in adaptive cardiac hypertrophy. Therefore, it is important to know how the cardiac function in these animals is. Aim. To analyze the effect of (-)-epicatechin falvonoid in the cardiac function of healthy mice *in vivo*. Methodology. The flavonoid Epi was administered at a dose of 1mg/kg body weight for 15 days every 12 hrs. The hemodynamic parameters were analyzed *in vivo* in the Epi-treated group and were compared with Ctrl mice. Hemodynamic measurements were performed according to the protocol described by Pacher et al., with modifications. The presence of heart failure markers (ANP and BNP) were determined by Western blotting. Results. Our results showed that heart rate, left ventricular end systolic pressure, stroke work, cardiac output, maximal and minimal change in rate of left ventricular pressure rise and left ventricular work minute decreased in the Epi group in comparison with a Ctrl group ($p < 0.05$, n = 6). The relaxation time constant increased in the Epi group in comparison with a Ctrl group ($p < 0.05$, n = 6), whereas other hemodynamic parameters, as left ventricular end diastolic pressure, left ventricular end systolic volume, left ventricular end diastolic volume, stroke volume, ejection fraction and end systolic elastance did not show changes between groups (n = 6). We found a statistically significant decrease in the ANP and BNP markers ($p < 0.05$, n = 6) in mice treated with Epi.

Conclusions. The hemodynamic analysis *in vivo* showed that Epi treatment induces cardiac adaptive changes, similar to those observed in athletes´ hearts.

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# Effect of supplementation of dehydrated pineapple core (*Ananas comosus*) in glycemic index and fiber content in a bisquet type bread

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**Abstract**

Pineapple is an important source of ascorbic acid, vitamins, minerals and dietetic fiber which includes polysaccharides, oligosaccharides, lignin and associated plant substances. Fiber also tend to promote physiological effects as a laxative and/or regulate blood cholesterol levels. In case of pineapple, the core is the part with a high fiber content. In industry, pineapple solid waste is discarded to the environment and the core contributes with 20% of the waste generated. The aim of this study was to evaluate the functional effect in bisquet when replacing wheat flour by dehydrated pineapple core; four substitutions were made at different percentages: 0%, 10%, 15% and 20%. To assess acceptability, 5 points hedonic scale was applied to 20 people between 17 and 60 years. Texture determination was performed by texturemeter. Also, quantification of dietary fiber and glycemic index prediction, was performed by Grandfield method. The most widely accepted sample corresponded to 15% bisquet replaced with pineapple core, with a rating of 9.73. This formulation also showed increase in total dietary fiber: 1.95g/100g versus 0.80g/100g of the original formulation (without pineapple). The substitution with 15% showed a decrease in glycemic index of 45 (low) versus 70 (high) of bread without pineapple. In conclusion we find that the best replacement of wheat flour by dehydrated pineapple was 15%, it had the best acceptability, and presented an increase of dietary fiber as well as a significant reduction in glycemic index.

# Effects of damiana (*T. diffusa* Willd. var diffusa) in diabetes lipids control

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The use of medicinal plants is a common practice among Mexicans for the empirical treatment of diabetes. *T. diffusa* Willd var. diffusa (damiana) is native from Northwest Mexico and used to treat diabetes that is a chronic degenerative disease with high morbidity and mortality rates caused by its complications. In this work diabetes mellitus was induced by a single intraperitoneal injection of streptozotocin in normoglycemic Wistar rats. Diabetic rats were treated with oral doses of aqueous extract from aerial parts of damiana to evaluate blood glucose, body weight, and lipid profile to try to explain the beneficial use of this medicinal plant in diabetes. Control animals received water as a vehicle in the same conditions. Glucose was monitored with Accu-Chek performance glucometer by Roche. Blood samples were collected with at least 8 h fasting after 8 weeks of starting treatment using the terminal procedure technique in glass tubes from posterior vena cava in all rats involved in protocols. Serum glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides were measured using commercially available biochemical kits. Our results have shown that damiana did not have the capacity to lower blood glucose, and it did not alter total cholesterol or LDL-cholesterol. However, it increased HDL-cholesterol and decreased triglycerides in the treated control group although no changes were observed in treated DM versus the non-treated DM groups. Another important observation was that the animals that received the extract tended to keep their body weight. With these observations, we concluded that damiana helps to mitigate the negative effects present in the diabetic people observed by lipids control.

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# Daily supplementation with fresh pomegranate juice increases paraoxonase 1 expression and activity in mice fed an atherogenic diet

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**PURPOSE:** Studies have concluded that pomegranate juice (PJ) consumption may delay the development of atherosclerosis by increasing the binding of high density lipoproteins (HDL) to Paraoxonase 1 (PON1), thus increasing the catalytic activity of this enzyme. PON1 is a calcium-dependent multifunctional enzyme synthesized in the liver and transported in plasma in association with HDL. PON1 also is able to hydrolyze Homocysteine (Hcy)-thiolactone which plays an important role in atherothrombosis. Decreased levels of PON1 are associated with higher levels of cholesterol and an increased risk for cardiovascular disease.

**METHODS:** We determine the effects of PJ on body weight, cholesterol, and triacylglycerols through five months of supplementation. Additionally, the effect of PJ on *pon1* gene expression in the liver was also measured by RT-qPCR as well as the activity in serum by a semiautomated method using paraoxon as a substrate.

**METHODS:** CD-1 mice were fed an atherogenic diet (1.25% (wt/wt) cholesterol, 0.5% (wt/wt) sodium cholate, and 15% (wt/wt) saturated fat) and administered by oral gavage with 300 uL of PJ containing 0.35 mmol total polyphenols in order to determine the effects of PJ through five months of supplementation.

**RESULTS:** By the end of the treatment period, PJ-supplemented animals had the lowest body weight. In addition, PJ significantly reduced serum cholesterol and triacylglycerol levels and also significantly induced *pon1* gene expression and increased the activity.

**CONCLUSIONS:** Overall, the administration of PJ suggests a possible use of PJ as a cardioprotective, particularly in cases of poor diet and overweight or obesity condition.

# Finding potential inhibitors of shikimate kinase from methicillin resistant *Staphylococcus aureus* through virtual screening.

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*Staphylococcus aureus* is a particularly virulent microorganism that is resistant to antibiotics, the main impact is due to methicillin resistant strains of S. aureus (MRSA) commonly found in hospitals, creating an increased necessity to develop a new antimicrobial therapy. In this context, shikimate kinase (SK) is an essential enzyme in the shikimic acid pathway involved in the biosynthesis of aromatic compounds in this bacterium. In this study, shikimate kinase from *S. Aureus* (SaSK) was characterized *in silico* and a virtual screening protocol was applied to identify new potential inhibitors. After the construction and validation of a SaSK 3D model, virtual screening, using Glide software, was performed into the active site of the enzyme. The “Drug like” subset of the ZINC database was employed. The structural analyses of the three molecules with the highest binding energy showed that compound ZINC34616948 made hydrogen bonds with Asp117, Glu57, Arg138 and Arg61; while ZINC03162994 formed hydrogen bonds with Asp37 and Asp117, a salt bridge with Asp37 and a π-π stacking with Phe60 were stablished. Compound ZINC70632388 made hydrogen bonds with Arg61 and Arg138, a salt bridge with Glu57, Arg61 and Arg138. These molecules could be potential inhibitors of SaSK and serve as a guide in the search of a new chemotherapy against nosocomial infections.

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# Characterization of a type 2 diabetes model in lactating Wistar rats

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The type 2 diabetes mellitus (DM2) model induced in neonatal rats by streptozotocin (STZ) is the most frequently used model for understanding the human diabetes. At long term periods, it has been shown that this model produces consequences in adult rats similar to humans; however, the metabolic starting conditions of this model, during the lactation, is not well defined and need to be characterized in order to understand the following consequences in adulthood. This animal model is mainly used for pathology studies in adults but, what are the consequences of the chronic hyperglycemia during lactation on the young rats? In the present study we characterized the hyperglycemic STZ-model induced in 2 days-old Wistar rats and analyzed at different times (7, 14, 21, and 28 days during lactation) the relationship between plasma glucose, body weight, cholesterol and triglycerides. The STZ induced group had higher average glycaemia from 30 to 90% above the normal group ($p<0.005$) and lower body weight ($p<0.05$) by the end of the lactation period. The cholesterol concentration was two-fold higher, and the triglycerides concentration four-fold higher in the STZ induced group. According to the parameters currently used for the diagnostic and control of diabetes in humans, the results shown in this study support the development of a DM2 model in lactating rats through the use of STZ. The increased blood glucose in the STZ-group was associated to pancreatic beta-cell destruction followed by partial regeneration, associated with a functional defect in glucose-stimulated insulin secretion, glucose intolerance and hyperglycemia. The excessive consumption of lipids and carbohydrates in the diet is one of the main factors associated with increased concentrations of cholesterol and triglycerides in adult stages; however in the infancy period it is regulated by nutrients in breastmilk. We also found that the higher the hyperglycemia during lactation, the higher the hyperglycemia in adulthood, reflecting the metabolic damage produced not only by the reduced capacity of insulin synthesis, but also by other phenomena such as the glucotoxicity derived from the metabolic programing during this stage of the development.

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# Extraction and characterization of protein fractions with nutraceutical potential from two species of insects of Tenebrionidae family: *Ulomoides dermestoides* and *Tenebrio molitor*.

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**Knowledge area:** Medicine, health and nutrition.

**Introduction** As a food source, insects are potentially nutritious, they are rich in protein, fat and certain amount of minerals and vitamins. Around of 1,900 species of edible insects are consumed worldwide. However, this number continues to rise as carried out further studies on this issue. Most of these known species are collected directly from the natural environment. Nevertheles there is al lack of information about the full nutraceutical potential of insects as a source of food.

**Objetive.** To extract, characterize and evaluate the nutraceutical potential of protein fractions obtained from two species of insect of family Tenebrionidae: *Ulomoides dermestoides* and *Tenebrio molitor.*

**Materials and methods.** The hatchlings are placed in a plastic container at a temperature of 25-30°C. Proximate analyses indicate the protein content, crude fiber, lipids, ash and nitrogen free extracts in the insect. An electrophoretic gel (SDS-PAGE) was performed to identify molecular weights of the proteins contained in the sample.

**Results.** Measured protein content of the tested insect species in this study was comparable with that of beef, chicken and fish. Furthermore, measured insect protein content was higher than that of lamb, pork, eggs, and milk, but lower in comparison to soy. Protein aqueous extracts of both insects were obtained by using two solvents: deionized water and phosphate buffer (PBS). It was confirmed that proteins were obtained by qualitative and quantitative tests, revealing that highest concentration was obtained with PBS extraction.

**Conclusion:** These results contribute to know about protein content on two species of Coleoptera. These initial screening of functional properties for coleoptera species showed a potential use for food industry.

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# FAT1 in Idiopathic Pulmonary Fibrosis

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The Idiopathic lung fibrosis (IPF) is a chronic, aging-related and lethal lung disease of unknown cause. In an effort to understand the pathogenic mechanisms of the illness, it intends that in the IPF the damage in the lung epithelium could unchain the proliferation, migration and activation of fibroblasts. The alveolar epithelial cells die in this microenvironment and the type 2 pneumocytes try to cover the damage (an characteristic of the IPF is the presence in great number of hyperplastics and hypertrophic epithelia which could be constituted by type 2 pneumocytes). Our work group carried out in previous years an analysis of multiple expression in which lungs were compared of patient with IPF *versus* controls (for hipersensitivity pneumonitis which is a different pathological entity), showing to a group of genes over-expressed in IPF. One of those genes is *Fat1*, which is a gene belonging to the cadherins superfamily; it is a vertebrate orthologue of *Drosophila*. Human *Fat1* was cloned from a T-leukemia cell line in 1995 and shown to encode a type I transmembrane protein with 34 extracellular cadherin repeats. *In situ* hibridation showed that *Fat1* expression was present in epithelial comparments, but high expression was found only in fetal as opposed to adult tissues. For this reason, the objective of the present work is to know that function carries out FAT1 in IPF and to evaluate the mechanisms that regulate the genic expression. For they were make it analysis of real time PCR in which were included to a group of samples controls and with hipersentitivity pneumonitis. The results show that overexpression of *Fat1* exists in lungs with FPI, not alone compared with hypersensitivity pneumonitis but also against versus normal lung. Immunohistochemistry with FAT1ab, showed positively stained in alveolar epithelial cells, mostly in bronchiolar epithelial cells, in an interesting way the sign of these cells is frequently observed in the basal part. Later on was carried out an analysis in an experimental model with alveolar epithelial cells A549 (ATCC) stimulated with TGF□1 to different periods: 3, 6, 9, 12, 24 and 96 hours, with the objective of observing the distribution in the expression of the gene *Fat1*; this type of experiments allows us to understand like the gene is regulated and which its importance can be inside the development of the IPF. A differential regulation was observed in the expression of the gene *Fat1* in the cells stimulated with TGF□1 at 3 and 6 hours; at the 3 hours it is sub-regulated and at the 6 hours it is overexpressed. This dates indicates us that differential regulation of the *Fat1* in IPF and a regulation mechanism by TGF□1 which work as chemical messenger in epithelial lung cells.

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# 2D-DIGE analysis of serum of Mexican patients with Type-2 diabetes in relation to their body mass index

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Proteomics is a powerful tool in clinical research and the past decade has resulted in several new findings of potential biomarkers of some diseases. These biomarkers reflect an important role in the patho-physiology and biological processes of diseases. In metabolic diseases such as type-2 diabetes (T2D) and obesity, different proteomic approaches have been reported; as a result the Human Diabetes Proteome Project (HDPP) has generated lists of proteins that are related to diabetes, which had been identified in different cell lines, animal models, and human fluids. However, most of these of reports have focused on European population and little is known about the proteins present in other populations such as Latin people and specifically in Mexican population. Considering that given the same dietary and lifestyle factors, some individuals may be more prone to develop T2D than others due to different genetic backgrounds, for example; individuals of Mexican ancestry are twice as likely as individuals of European ancestry to present T2D. For these, it will be of great interest to try to understand the pathogenesis of the disease in the Mexican population. Hence, the aim of the present study was to analyse the serum proteomic profile of Mexican patients with T2D subgrouped according to their body mass index of each patient. Five categories were defined: normal weight, overweight, obese class I, obese class II/III, and control group of healthy volunteers.

Blood samples from all groups were collected under informed consent. Detection of 10 diabetes-related peptides using MAGPIX system was carried out in order to validate their diabetic and obesity condition. Serum proteins were fractionated using MARS14 affinity columns and high-abundant and low-abundant proteins were labelled with CyDyes for DIGE (Difference Gel Electrophoresis). Labelled proteins were separated on 24 cm gels, scanned and analysed with DeCyder 2D-DIGE analysis software (v7.2). Differentially accumulated protein spots were excised from preparative gels, digested for LC-MS/MS analysis, and identified using MASCOT (v2.5) search engine tool. Proteins of interest (POI) were defined as those that showed in the extended data analysis a behaviour through the groups related to a variant on the study (Diabetes, Obesity or Diabetes-Obesity related protein). Validation of four of the best POI selected, was carried out by Western blotting. Our results shows evidence of new diabetic related proteins in serum that has not been previously reported by the HDPP lists, those proteins might be related to the ethnicity differences, representing new candidate biomarkers for the disease in Mexican population.

# Antimicrobial and Antibiofilm effect of Flavonoids in Periodonto pathogens

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Periodontal disease ranges from simple gum inflammation to serious periodontitis. That results in major damage to the soft tissue and bone in oral cavity that leads to tooth loss. This is caused by diverse periodontopathogens and their hability to produce biofilms. Flavonoids are a diverse group of plant metabolites with over 10,000 compounds that have been identified until now. They have several important antioxidant, antiviral and antibacterial properties. Among foods that provide large amounts of these substances there are: citrus fruits, blueberries, blackberries, onions, peppers, a variety of teas, and also oregano and parsley. In this study we performed Kirby Bauer test to determinate the antimicrobial effect against different pathogens like: *Actinomyces naeslundii, Escherichia coli, Enterococcus faecalis, Streptococcus sanguinis, Actinomyces viscosus, Candida albicans, Staphylococcus aureus and Aggregatibacter actinomycetemcomitans.* The zone of inhibition indicated actual antimicrobial effect from the tested flavonoids and mixtures of them (Morin, Quercetin, Catequin, Luteolin and Naringin) at different concentrations. Moreover, biofilm growth was performed in BHI medium and treated with Catequin, Quercetin and Morin at a 1 mg/mL concentration. Biofilms were treated during their formation process and also after it was consolidated. The results of the performed biofilms showed us that flavonoids have antibiofilm effect during their development, and also a null effect when it is consolidated. This results showed that flavonoids have an antimicrobial effect if it's applied during the bacterial growth, so they can work preventing periodontal disease.

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# Expression of Opioid Grow Factor Receptor (OGFr) and Transient Receptor Potential Vanilloid 1 (TRPV1) In a Rat Alkali-Burned Cornea Model.

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**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 and the histopathology after alkali-burned cornea injury. **Methods:** We used Wistar rats of both sexes of 200-300 grams of weight under general anesthesia with *zoletil 50* 25mg/Kg. Alkali burns were generated in both eyes with 6mm of diameter filter paper soaked with NaOH 1N, then placed 45 seconds on each cornea, next after rinse the eye with saline solution, ophthalmic strips with sodic fluorescein were used as indicator of damage under cobalt blue light. Corneas were collected at 0, 24, 48 and 72 hours after alkali-burned injury. Control cornea group were kept intact and collected in the same time course. The corneas were placed in paraformaldehyde or TRIZOL®reagent. OGFr and TRPV1 expression was examined by using endpoint RT-PCR, tissue integrity was evaluated by hematoxilin/eosine (HE) preparations. **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 by PCR, detecting both in controls and burned corneas but some changes in the levels of expression. **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 have lower levels of expression in the damaged corneas.

# Endothelial activation mediated by tumor soluble factors secreted by breast cancer cell lines with low and high metastatic potential

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**Background:** Endothelial activation includes an increased expression of adhesion molecules on the apical membrane and an increase in vascular permeability, which contribute to the extravasation process of circulating cells. Some cancer cell lines release soluble factors that activate the endothelium in vitro, with no interaction between cells. This has been defined as premetastatic state, and it could be associated with extravasation of circulating tumor cells. We propose that content of tumor soluble factors (TSFs) secreted by breast cancer cell lines with diverse metastatic potential is different, and differentially induce endothelial activation and subsequent recruiting of circulating cells. **Material and methods:** Culture media of breast cancer lines with low (T47D) and high (ZR75-30) metastatic potential was collected and used to obtain the TSFs. Primary human umbilical-vein endothelial cells (HUVEC) were stimulated with TSFs (10 µg/ml) and polymyxin B. Afterwards, tritium labeled U-937 cells were added, so that they could adhere only to the activated HUVEC, and radiation was quantified. Evans Blue dye was administered intravenously on Swiss Webster mice. Afterwards, TSFs (16 µg/ml) were administered intradermally on the dorsal area; an increase of vascular permeability allows the extravasation of the dye. The extracted dye of the dissected skin was quantified by spectrophotometry at 620 nm. **Results and discussion:** Only the TSFs secreted by ZR75-30 cell line (TSF-ZR75-30) activated the endothelium in vitro an in vivo to similar levels of the activation caused by the positive controls, TNF and VEGF respectively. Endothelial activation induced by TSF-ZR75-30 was reduced by polymyxin B, approximately by 30%. Polymyxin B is a selective inhibitor of PKC, on its presence endothelial activation is reduced but not completely abated because PKC is part of the signaling pathways of only some components of ZR75-30, for example, IL8 and VEGF. The endothelium can be activated by the other components independently of PKC. **Conclusions:** TSFs secreted by the cell line with high metastatic potential (ZR75-30) activate the endothelium in vitro and in vivo; whilst TSFs secreted by the low metastatic cell line (T47D) do not activate the endothelium. PKC is involved on the endothelial activation induced by TSF-ZR75-30. Determination of the key components of TSFs and its signaling pathways will allow generate new diagnosis biomarkers.

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# PCR identification for the four most common *Candida* species in nosocomial infections

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Candidemia is a widespread nosocomial infection in the bloodstream caused by several species of fungal pathogens of the genus *Candida*. Candidemias are associated with high mortality rates. The most frequently found species worldwide is *C. albicans*, followed by *C. glabrata* and *C. tropicalis* and finally by *C. parapsilosis*, although the relative frequency of each of these species varies with the geographic zone from which the samples are obtained. The treatment for these infections depends on the particular species causing the infection and the anatomical site affected, as some species display resistance to particular antifungals. Therefore, timely identification of the species is important to start early the appropriate therapy. The conventional methods for diagnosis of *Candida* species in hospitals are usually slow and imprecise, thus the development of new faster and more accurate molecular methods is required for a better patient outcome.

We have developed a molecular identification method for the four most common *Candida* species based on the amplification by PCR of species-specific sequences of *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. parapsilosis* . We analyzed 94 positive isolates of *Candida* (identified at the hospital by VITEK®) from 58 patients from the Instituto Nacional de Ciencias Médicas y de la Nutrición Salvador Zubirán (INMNSZ). We found that 90.43% of the samples (85 isolates), were positive for one of the four species that we can detect..The most frequent species was *C. glabrata* with 24.47% (23 isolates), followed by *C. albicans* and *C. tropicalis* with 23.4% (22 isolates) each. However several of these samples are derived from single patients taken at different times during the hospital stay. Considering these samples as just one appearance of that species, the most prevalent species were *C. albicans* with 27.59% (16 isolates), followed by *C. parapsilosis* and *C. glabrata* with 25.86% (15 isolates) respectively. The isolates we could not identify with our method, were identified by the VITEK® method at the hospital as different *Candida* species from the 4 we studied (i.e. *C. krusei*, *C. guilliermondii* etc) showing that our method does not give false positive results. We are now establishing the conditions to use our method with qPCR to increase the sensitivity.

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# Development of functional gummies using reduced moisture xoconostle (*Opuntia joconostle*) pulp

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## Abstract

The xoconostle is a type of tuna from a variety of cactus (*Opuntia joconostle*) with sour flavour, its mesocarp-endocarp is the edible part that contains mainly sugars, dietary fiber, and phenolic compounds. In this work the bioactive compounds of xoconostle mesocarp-endocarp were used as a functional ingredient, in order to incorporate it into the formulation of a jelly gummies. Gummies were made varying the concentration of the jelly by reduced moisture pulp xoconostle: 0% (control), 40% y 50% replacement: being constant the other ingredients. The following physical analyses were performed: colour index, acidity, and pH; sensorial evaluation: acceptance level using a 5-point hedonic scale with untrained evaluators; proximal chemical analysis; concentration of total polyphenols (Folin-Ciocalteu) and antioxidant capacity (DPPH). The intrinsic property of xoconostle increased the acidity content, which had influence in the characteristic flavour of the gummies. The untrained evaluators gave a rating of "likes" to the gummies with 40 % concentration. The xoconostle pulp had an increase in the content of minerals, protein and fiber with xoconostle gummies, being 2 to 3 times higher in comparation with the control. Also, the concentration of phenolic compounds and its antioxidant capacity were higher than the control, which were nil. The best sensorial attributes and the most antioxidant capacity of gummies was using a transparent packaging. In conclusion, the pulp xoconostle improved the sensorial characteristics, as well as the antioxidant capacity of the gummies.

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# Changed of platelet activity in patients with hypothyroidism

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**Introduction.** Hypothyroidism is a condition resulting from the decrease in the effect of thyroid hormones tissue level; where the most frequent cause is the decrease in the synthesis and secretion of the same, and sometimes peripheral resistance to thyroid hormones. It is recognized the influence of thyroid dysfunction in metabolic processes, but its relationship with platelet activity has not been deeply investigated. To analyze this pathology propose to compare the response in platelet aggregation agonist such as ADP and thrombin, and intracellular calcium release, hypothyroid patients with and without antithyroid antibodies, in order to determine if alterations in thyroid function can be associated with changes in platelet activity.

**Material and methods. Platelet aggregation with adenosine diphosphate (ADP).** Rich plasma was used (PRP), obtained of healthy patients with hypothyroidism fasting. Immediately after collection the blood samples were centrifuged with 10% dextrose containing citrate anticoagulant at pH 6.5 at 1000 rpm for 10 minutes at room temperature. The PRP was adjusted to 200,000 platelets with pH 6.5 Buffer CGS and response of platelet aggregation with 0.58µM, 1.17 uM 2.34 uM and ADP was measured. **Platelet aggregation with thrombin.** PRP was obtained, immediately after collection the blood samples were centrifuged with 10% dextrose containing citrate anticoagulant at pH 6.5 at 1000 rpm for 10 minutes at room temperature. The PRP was centrifuged obtained with EDTA Buffer pH 6.5 CGS-1: 1 v / v for 15 minutes at 1000 rpm. Platelet package obtained was suspended in 3 mL of pH 6.5 Buffer CGS and centrifuged for 12 minutes at 1000 rpm. Platelet package obtained was adjusted to 200,000 platelets with pH 6.5 Buffer CGS and response of platelet aggregation with an end unit of thrombin was measured. **Measuring intraplatelet calcium.** To measure the intracellular calcium concentration, washed platelets and aggregation by thrombin, were labeled with fluo-3 a.m. acetoxymethyl ester (4µM to 37 ° C for 30 minutes. Using a Perkin-Elmer LS 55 spectrofluorometer, she underwent a scan of fluorescence intensity against time (220 seconds), the samples were excited at 505 nm and fluorescence emission was collected at 530 nm. with these parameters readings mobilization of [Ca2 +] i were performed with a unit thrombin. the Ca2 + concentration was calculated intraplatelet from fluorescence measurements, using the equation of Grynkiewicz et al.
**Results.** Patients with negative antithyroid antibodies exhibit normal platelet aggregation with thrombin while those with positive antithyroid antibodies do not add thrombin. ADP platelet aggregation was normal. Intraplatelet calcium concentration in hypothyroid patients is tripled compared with control subjects. **Conclusion.** Platelet activity is modified in patients with hypothyroidism, and the presence of positive antithyroid antibodies could affect platelet aggregation through the pathway of thrombin.

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# Effects of insulin and interleukin 10 (IL 10) in migration and proliferation process on HeLa, C33A and HaCat cells.

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**Introduction:** Cervical cancer is one of the neoplastic diseases considered as the second leading cause of death in women worldwide. There have been described several genes and proteins related to the regulation of cell proliferation in different stages of cancer progression. Diverse studies report that insulin stimulates growth and proliferation in an assortment of cell lines of human breast cancer. Also it has been shown that insulin can activate signaling pathways, associated with proliferation and migration in cervical cancer cell lines, SiHa ( HPV16+). Recent research suggests that interleukin-10 (IL-10) is secreted by cancer cells, including cells from cervical cancer, but it is no yet known what kind of effect has in this type of cells; however, it is known that IL-10 takes a role in proliferation and survival of myeloid cells by increasing cell anti-apoptotic activity. The aim of this work was to analyze changes in migration and cellular proliferation in HeLa, C33A and HaCat cells in response to insulin and rhIL-10. **Metodology:** In this in vitro study, HeLa (VPH18+), C-33A (VPH-) cell lines, and HaCat cell line, were stimulated with different concentrations of insulin and rhIL-10 (10, 50, 100 nM/5, 10 y 50 ng/mL). Changes in cellular proliferation were evaluated via MTT essay and by cell counting in Neubauer chamber. Migration essays were carried out by wound healing technique; data were analyzed with TScratch software. **Results:** HeLa and C33A cells treated with insulin at different concentrations showed lower cell proliferation than the control group, being more evident at 50 and 100 nM. HeLa cells stimulated with 5 and 50 ng/ml rhIL-10, displayed a significant decreasing in cell proliferation ($p<0.05$) at 48h in comparison with the control group. In C33A cells treated with rhIL-10 it was observed a significant decreasing in proliferation. HaCat cells stimulated with insulin or rhIL-10 showed no significant changes in proliferation. Migration of HeLa cells increased after 48 hour of stimulation with 100 nM of insulin, while HaCat cells showed an increment in migration after 48 hours, but with a lower dose of insulin (10 nM). In C-33 cells stimulated with insulin, no differences in migration were observed among the different doses tested. In HeLa and C-33 stimulated with rhIL-10 no changes in cell migration were observed in comparison with the control group, while HaCat cells showed an increment in migration after stimulation with 50 ng/mL of rhIL-10 at 24 hours. **Conclusion:** These results suggest that HeLa and C-33 cell lines respond to stimulation with insulin and rhIL-10 exerting an inhibiting effect on proliferation. While in HeLa and HaCat it was observed an increase in cell migration in response to these stimuli.

# Antineoplastic capacity of *Moringa oleifera* extract through regulation of pyruvate dehydrogenase complex in a breast cancer murine model.

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Cancer cells differ from normal cells due to the metabolic alterations presented. One of them is the decrease in the pyruvate dehydrogenase complex (PDC) activity, as a result of its phosphorylation by the action of pyruvate dehydrogenase kinases (PDKs). This makes it a potential molecular target in the search for new therapeutic alternatives against cancer. On the other hand, *Moringa oleifera* represents an alternative for cancer therapy, as a source of wide variety of polyphenols with antitumoral activity. Therefore, the objective of this work was to assess the antineoplastic capacity of a hydroalcoholic extract of *Moringa oleifera* leaves and its effect on the PDC regulation in a breast cancer murine model. To this end, female Sprague Dawley rats were induced with a single dose of N-nitroso-N-ethyl urea (ENU), 250 mg/kg body weight, at the age of 48-51 days. The groups were divided in control, untreated cancer, cancer treated with *Moringa oleifera* (daily dose of 500 mg/kg body weight for one month after two months of induction). A morphometric analysis and histologic diagnosis was performed in order to evaluate the effect of the extract on tumor development. The group treated with *Moringa oleifera* showed a decrease in the incidence, number of tumors per rat, and size of tumors. Also, modulation of PDC activity was observed in the treated group compared to the control and untreated group. Finally, a docking study was conducted to analyze the molecular interactions between PDC and phytochemicals of *Moringa oleifera.* Data showed that quercetin, catechin, luteolin and cryptochlorogenic acid had the highest binding energy in PDC, which indicates that these compounds could be potential ligands of PDC and be involved in their regulation.

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# THIAMINE DEPRIVATION PRODUCES SIMILAR METABOLIC AND GENOMIC EFFECTS AS BIOTIN DEFICIENCY

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In previous findings, we find in biotin-deprived (BtDEF) rats, a deficit of ATP associated with reduced flow through the TCA cycle due to reduced anaplerosis caused by pyruvate carboxylase (PC) deficiency, with electron transfer chain (ETC) complex IV (cytochrome c oxidase) deficiency and decreased oxidative phosphorylation, and with diminished mitochondria mass due to toxicity by acyl CoA compounds and raised signaling through a novel mitophagy signal transduction pathway triggered by inflammation. Also, in BtDEF as a result of a direct effect in the AMP dependent kinase (AMPK) insulin sensitivity is enhanced. In other hand we observed changes in the carbon metabolism genes, reporting a diminution of mRNAs for glucose use and lipogenesis and a rise in fatty acid oxidation and gluconeogenesis mRNAs. This finding are similar to those found in biotinidase knockout mice, being this a key enzyme for the reuptake of biotin. In order to know if these changes are specific to vitamin deficiency or are a reflex of a general alteration of the ATP machine, recently we have begun similar studies with thiamine deficiency (TD). Thiamine (B1 vitamin) is a cofactor for three critical dehydrogenases: pyruvate dehydrogenase complex catalyze pyruvate to acetyl CoA, a ketoglutarate to succinate CoA, branched chain ketoacid dehydrogenase and tranaldolase in the pentose phosphate pathway. Biotin is a carboxylase protesic group an Thiamine a dehydrogenase cofactor, both essential for the proper functioning of metabolism.

The experiments were performed in four weeks old C57BL/6 mice. They were fed a thiamine deficient diet and killed in weekly intervals during the first three week. TD was evaluated measuring thiamin pyrophosphate via HPLC. mRNAs were determined with qRT-PCR. Several metabolic parameters were measured: glucose, lactate and amino acids in blood, insulin, free fatty acids triglycerides and cholesterol in serum, hepatic glycogen in tissue. The metabolic an genetic effects of thiamin deficiency are mainly similar to the ones in biotin deficiency. This studies gives new information to the role of nutritional cofactors required to ATP generation and to the metabolic genomic control. This information can be relevant in energy related disorders.

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# Alzheimer´s Disease: In The Eye?

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**Introduction**: Murine transgenic models of Alzheimer's disease (Tg-AD) and aging models have been used to analyze the presence of β-amyloid precursor protein (β-APP), Aβ 1-42, as amino truncated species like Aβ3-42 and Aβ 11-42 peptides deposition in the brain. These peptides have been categorized as citotoxic and the principal markers in AD. Besides, they have been involved in other pathological mechanisms during the development and progression of AD. Recently, the ßA42 peptide has been involved in vision loss. The presence of multiple ßA42 reservoirs in the eye, specially in the retina environment, induces pathologies that leads to deficient vision and blindness. Pathologies like Age-Related Macular Degeneration (AMD) and cataracts may contribute to the local inflammatory events involved in the formation of local deposits of lipids and beta amyloid peptide called drusens, atrophy of retinal pigment epithelium, lens degeneration and photoreceptor cell death.

However these mechanisms have not been totally demonstrated and our current research is being performed in order to probe if amino truncated species are involved in vission loss in aging and AD.

**Results**: In this report, we have studied the deposition levels of β-APP, Aβ 42, Aβ3-42 and Aβ 11-42 in retina in a 6 months old and 24 months old of 3xTg-AD model as well in 6 months old and 24 months old aged symptomatic mice. Western blot and Immunofluorescence using specific antibodies for each beta amyloid peptide were performed in protein extracts of isolated retinas and in histologic slides of the two models respectively

The results show the significant deposition of the different toxic peptides in the retina at 24 months old principally.

**Conclusions**: These may be a useful model to study the mechanisms of amyloid mediated vision degeneration in AD and aging. More studies are needed in order to demonstrate the production mechanisms of these peptides in the eye of these two models.

Interestingly, there is not enough information about the presence of the N-truncated species of beta amyloid peptides in the eye. The big question is: Why? Isn´t there relevance? Or only has not been studied? More research in this topic is urgently needed in order to improve the quality of life of patients with AD and aging.

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# *In vitro* validation of differential gene expression for Acute Leukemia subtypification in Baja California Sur

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**Introduction** Leukemia is a progressive, malignant disease of the blood-forming organs, marked by distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow. Based on the speed of progression, this disease may be classified as either acute (fast) or chronic (slow), and depending on the types of cells involved, it may be characterized as lymphoid or myeloid. International reports have shown that Mexico has the highest mortality rate due to leukemia in Latin America, and in 2008 Baja California Sur had the 4th highest rate nationwide. In practice, bone marrow and peripheral blood samples are used to make a diagnosis based on the initial morphologic and immunophenotypic findings. However, molecular techniques are often necessary in order to further classify the type of leukemia. In a previous investigation, an algorithm able to correctly classify subtypes of acute leukemia was developed, where cytoskeletal genes, *ZYX, MacMARKS* and *GPI*, whose expression level varies significantly, were selected. Therefore**,** using Molecular Biology techniques is an option for the identification and analysis of gene signatures associated with the region of Baja California Sur, which will improve the diagnosis and treatment of leukemia.

**Methodology** 25 archived blocks of Formalin-fixed Paraffin-embedded (FFPE) bone marrow samples were obtained. Deparaffinization was done using xylene and ethanol, and peripheral blood samples were fractionated by differential centrifugation. RNA isolation was performed using Guanidine-Thiocyanate and phenol-chloroform technique, and quantification was done through spectrophotometry. Constitutive genes and differential expression genes oligonucleotides were designed. RT-qPCR will be accomplished using EvaGreen® dye, and statistical analysis will be achieved to calculate relative changes in gene expression experiments using the $2^{-\Delta\Delta Ct}$ method.

**Results and conclusions** Protocols for blood fractionation and deparaffinization of bone marrow samples were suitable as the concentration of RNA obtained was between 100 and 400 ng/µl, varying considerably with respect to sample content. Additionaly, $^{260}/_{280}$ purity ratios were within the known ranges established, between 1.8 and 2, which is an indicator of RNA quality for the downstream application. cDNA synthesis was performed, obtaining an efficiency of 50 to 95 %. *TUB, HPRT, ACTB*, and *GAPDH* will be tested to identify the appropriate gene for using as endogenous control and normalize the expression of *ZYX, MacMARKS* and *GIP* in the statistical analysis to succeed *in vitro subtypification* of acute Leukemia.

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# Evaluation of the antifungal activity of imidazo[1,2-a]pyridine compounds on *Fusarium oxysporum* f. sp. *lycopersici*

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The tomato *(Solanum lycopersicon* L.) is one of the most consumed vegetables in the world, the demand increases continuously and with it their cultivation, production and trade. In the records of the Food and Agriculture Organization (FAO) it is ranked that China as the leading producer of tomato with 50 million tons, while Mexico ranks tenth place with 3.2 million tons per year in 2013. The main producing states in Mexico are; Sinaloa, Baja California, San Luis Potosi, Sonora, Nayarit, Morelos and Michoacán. Production capacity of the tomato at national level is 41.67 tons per hectare.

One of the most important diseases that affects tomato root is the fusarium wilt, caused by the fungus *Fusarium oxysporum* f. sp. *lycopersici*, affecting over 50% of the tomato fields. So it is of interest to test compounds having activity against this organism, as in the case of imidazo[1,2-a]pyridine compounds which are reported to have antifungal activity against *Candida albicans, Candida parapsilosis, Aspergillus flavus* and *Microsporum gypsuem*.

The tests were performed using imidazo[1,2-*a*]pyridine having in 4'-position fluorine, methyl, methoxy and bromine moieties through the Poisson plate technique using PDA with concentrations of 25, 50, 100, 150 and 300 µg/mL for the compound tested and two negative controls, distilled water and DMSO. The DMSO control was used because it is the solvent employed to solve imidazo[1,2-a]pyridine derivatives.

The obtained results from above test suggests that the compound with the greater antifungal activity is the one with the methoxy substituent, giving an inhibition percentage between 65.7, in the third day, and 29, in the sixth day, using a concentration of 300 µg/mL. The compound with the less antifungal activity is with the fluorine substituent moiety, giving an inhibition percentage between 16.2, in the third day, and 6.2, in the sixth day, using a concentration of 300 µg/mL. However, the methyl substituent with a concentration of 100 µg/mL improve the fungal growth between 6.8, in the fourth day, and 0.85, in the sixth day. A possible explanation for the highest inhibition percentage observed for the compound functionalized with methoxy group could be its electrodonating nature considering that the compound having the fluorine moiety (an electrowithdrawing group) was less effective.

# Incidence Of Atherogenic Risk Factors In A Population Of The State Of Tabasco.

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Area 12: Clinical Biochemistry

Atherosclerosis (AT)develops significant changes in the physiology of the arteries, since areas of calcification are formed in the vessel wall, no loss of elasticity and lipid accumulation, which together with other molecules causing malfunction and a decrease in vessel lumen artery. AT is a disease that develops in normal conditions and can be influenced by different factors, such as: high cholesterol, hypertension, sedentary lifestyle, smoking, elderly, among others. This is an exploratory retrospective cross-sectional study of the incidence of risk factors for the development of chronic degenerative diseases such as atherosclerosis in the municipality of Cunduacan the state of tabasco, 240 samples which were determined lipid profile (triglycerides, cholesterol HDL, LDL and IA), and anthropometric parameters (age, gender) to assess levels of risk of developing atherosclerosis, we found an incidence of 66.25% female against a 33.75% males, age where this type of performed laboratory tests was in the range of 36 to 69 years with 61.25%, followed range 18-35 years with 22.91%. We detect in 26% of people an index of 200- 240mg / dl cholesterol and 13% greater than 240mg / dl, in terms of triglycerides was 19% on average from 150 to 199mg / dl and 31% in more than 200 mg / dl in HDL levels only 8% of all people are at high risk for LDL levels only 1% have high levels. As in iatrogenic index are different levels in terms of male and female gender check at 0% in male have high risk levels with the development of atherosclerosis and 9% in women.

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# Matrix Metalloproteinase (MMP)-28 increases proliferation rate and migration of lung alveolar and bronchial epithelial cells, and localizes in the nuclei of alveolar epithelial cells in Idiopathic Pulmonary Fibrosis.

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Matrix Metalloproteinases (MMPs) are a family of zinc-dependent enzymes that not only modify the extracellular matrix (ECM) and the ECM bioactive molecules, but also play important roles in diverse processes. MMPs are secreted or membrane-bound enzymes, and recently some of them have been localized in mitochondria and even inside the nucleus.

Idiopathic Pulmonary Fibrosis (IPF) is a chronic, progressive and generally lethal disease of unknown etiology where the aberrant activated alveolar epithelial cells (AEC) induce the expansion and activation of the fibroblast population, along with excessive synthesis of ECM and destruction of the lung architecture. Microarray assay revealed that some MMPs are upregulated in IPF patients. One of them is MMP28, the last member of the family, a protein that had not been studied in human lung.

Our results show that MMP28 is increased in two different cohorts of IPF patients versus controls. By immunohistochemistry and immunofluorescence of lung biopsies of IPF patients, we found that MMP28 is expressed by the AEC and bronchial epithelial cells (BEC), but interestingly it localized in the nuclei of AEC.

In order to study the function of this protein, primary BEC and A549 cell line were silenced while AEC and rat AEC were transfected with MMP-28-mycDDK. Results show that silencing of MMP28 in BEC and AEC decreased proliferation rate and delayed wound closing, while overexpressing of MMP28 showed the opposite: faster proliferation rate and accelerated wound closing.

*In vitro*, MMP28 localizes in the nucleus of AEC under certain conditions. *In silico* analysis revealed a probable Nuclear Localization Signal (NLS) in MMP28, which could represent a mechanism for getting inside the nucleus. Site-directed mutagenesis (K→Q) in the NLS exhibits contradictory results. Future experiments include an approach to the probable nuclear function of MMP-28. This research will help to understand the role of MMP28 in IPF.

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# Vasculogenic mimicry is inhibited by angiomiR miR-204 in breast cancer

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**Background:** The mechanism by which tumor cells obtain nutrients and oxygen is the classical angiogenesis, which is defined as the formation of new blood vessels from pre-existent vasculature from endothelial cells. However, there is a distinct pathway that is still understood denominated vasculogenic mimicry (VM) defined as the capacity of tumors cells to forms 3D tubular channels, independent of endothelial cells in response to starvation and hypoxia. This novel phenomenon occurs in diverse types of cancer and stimulates the activation of genes that enable the remodeling of the extracellular matrix and arrangements of the tumor cells which develop 3D tubular structures that allows the perfusion and nutrients dissemination into the tumors. VM is characteristic of highly metastatic and aggressive tumors, which results in worse prognostic and poor survival. Recent reports indicate that classical anti-angiogenic therapies commonly fails may be by the presence of VM, thus it represents a novel target in cancer therapy. Remarkably, microRNAs may modulate the genes responsible for VM. However, only two miRNAs have been reported in cancer VM

**Results:** Here we evidenced the existence of vasculogenic mimicry in triple negative breast cancer MDA-MB-231 cells. The formation of 3D tubular channels indicative of vasculogenic mimicry were observed after 12 h of MDA-MB-231 cultures in matrigel under hypoxia condition. Moreover, the co-culture of MDA-MB-231 cells with HUVEC endothelial cells resulted in the increased formation of tortuous and irregular tubular structures. As previously, we found that miR-204 was able to inhibit classical vascular angiogenesis, here we tested if restoration of miR-204 expression in MDA-MB-231 cells also impairs vasculogenic mimicry *in vitro*. Our data showed that vasculogenic mimicry was abolished in mono and co-culture with HUVEC cells after hypoxia when miR-204 transfected in MDA-MB-231 cells. Western blot assays, in progress, will allows to identify the proteins involved in the miR-204-dependent inhibition of vasculogenic mimicry in breast cancer cells.

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# Identification of oral bacteria associated with periodontal disease in patients on chronic hemodialysis.

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Cardiovascular complications are the leading cause of death in population with Chronic Kidney Disease (CKD). There has been more evidence of systemic inflammation as a non-conventional risk factor for accelerated atherosclerosis in this population. Periodontitis has been identified as a risk factor for Cardiovascular Disease (CVD), through chronic systemic inflammation. Chronic subgingival infection with predominantly Gram-negative anaerobic bacteria can stimulate an acute- phase response and induce to continuous production of circulating cytokines, which that may increase inflammatory activity in atherosclerotic lesions, potentially increasing the risk of cardiac and cerebrovascular events.

Previous reports have demonstrated an association between periodontal disease and elevated C-Reactive Protein (CRP) levels in patients with chronic hemodialysis (HD).

The aim of this study was to determine the prevalence of a group of oral bacterial species from periodontal pockets and the gingival crevice of patients on chronic hemodialysis with periodontal disease to evaluate the association between the presence of these bacteria and the systemic inflammation.

Sixty-three patients with normal oral status/mild periodontitis, 22 patients with moderate periodontitis, and 26 patients with severe periodontitis were analyzed. Average age of study population was 42.9 years (±17.8 years), with a mean time on HD of 27.1 months (±30.5 months), 57 (51.3%) of patients were males, and 51 (45.9%) had diabetes. Samples were collected to determine the presence of *s. mutans, s. mitis* and *s. sanguinis* by PCR.

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# Comparison of commercial kits for molecular biology research.

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A critical step for studies on clinical, experimental or environmental samples is the use of adecuate DNA extraction kits or methods, good quality PCR kits and DNA staining reagents. We compared non-commercial DNA extraction method with commercial, also we compared two kits for PCR and two reagents for DNA staining. Our evaluation includes: time consumed, cost per reaction, quality and quantity.

Bacterial DNA extraction was the first comparison. We compared a Phenol:Chloroform:Isoamyl Alcohol based method (Gober´s et al), which, despite being cheap, produce harmful wastes and the DNA is not totally purified and the Bacteria DNA Preparation Kit (Jena Bioscience GmbH-Ref; PP-206), which is based on non harmful solutions and guarantee minimal DNA fragmentation.

The second comparison was between two commercial kits for PCR, we selected the kits based on their cost per reaction. The first kit is a premixed ready-to-use solution containing Taq DNA polymerase, dNTPs, MgCl2 and reaction buffers; also it contains two dyes and the reactions assembled with this kit have sufficient density for direct loading onto agarose gels, and was compared with the Red Load Taq Master / high yield (Jena Bioscience GmbH; Ref. PCR-106), which is a similar master mix but it contains additionally (NH4)2SO4, Tween-20, Nonidet P-40, red dye, gel loading buffer and stabilizers.

The last comparison was between reagents for nucleic acids staining. We compared Ethidium Bromide with EvaGreen® Fluorescent Gel Stain (Jena Bioscience GmbH; Ref. PCR-256)

For the bacterial DNA extraction, Gober´s method showed higher DNA concentrations (quantified with Nanodrop 2000), but the 260/280 ratios were unacceptable, instead, the kit showed minimal concentrations with 260/280 ratios above 1.8. We check the DNA quality performing a PCR to amplify a fragment of 16S gene, only kit samples amplified. Additionally to the quality of the DNA obtained using the kit, the time of DNA extraction is reduced.

The Red Load Taq Master / high yield (RL) gives superior amplification results in comparison with the other commercial kit, having the same cost per reaction. We tested several samples with different DNA concentrations, lower DNA concentration PCR products only were observed with the RL kit.

EvaGreen® Fluorescent Gel Stain, provide a high fluorescence that allow the detection of lowest DNA amounts. It is loaded with the sample, so it minimizes the unespecific staining, that is very common with Ethidium Bromide. Ethidium bromide may present a hazard if it is poured down the drain untreated or placed in the trash, instead,EvaGreen is safer, so it can be discarded directly in the drain.

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# Early activation of autophagy in response to DNA damage caused by Irinotecan in mammal cells

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Macroautophagy (referred as autophagy hereafter) is an evolutionarily conserved process in which some macromolecules and organelles of eukaryotic cells are degraded through the fusion of double-membrane vesicles called autophagosomes with the lysosomes. This catabolic pathway is misregulated in various pathological conditions like cellular senescence, neurodegeneration, and cancer. These diseases are also related to an accumulation of DNA damage and therefore, genome instability.

Defects in DNA single strand break repair is one of the most common genomic lesions and a link has been found between this type of damage and several neurodegenerative disorders. Interestingly, cells without FIP200 (an essential autophagy protein) accumulate major levels of DNA damage generated by camptothecin (a topoisomerase I blocker that produces single strand breaks). This cells also failed to repair the DNA and consequently, had increased sensitivity to cell death. In this work we hypothesize that single strand breaks would activate autophagy during early DNA damage in normal cells (mouse embryonic fibroblasts, MEFs) and that this response could vary in a cancer cell line (A549, lung adenocarcinoma) due to the different functions that autophagy has in cancer.

Through the evaluation of DNA damage induced by Irinotecan (a camptothecin derivative), no differences were found between the dose and exposure time required to generate DNA single strand breaks and to repair them in both MEFs and A549. By analyzing the kinetics of autophagic proteins, we observed that while in MEFs, autophagy was activated from the first time of exposure to Irinotecan and continued active during DNA repair; the cell line A549 did not activate autophagy neither during DNA damage nor during DNA repair. Our study indicates that autophagy is activated during early stages of DNA damage and through the repair process in normal cells, but not in A549 cancerous cells.

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# Omega-3 fatty acids and diabetes: effects on lipid metabolism and biological membranes.

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The fatty acids are known for their function in several processes such as energy storage, electrical and thermal isolation, part of signaling molecules and/or second messengers, precursors for synthesis of eicosanoids, regulators of gene expression, and they are the most important building blocks of biological membranes. It is likely that the variety of functions of lipids, besides their physical and chemical properties, confers them the chance of interaction with several cellular component for controlling the metabolism. The omega-3 fatty acids (ω3) are part of the group of essential polyunsaturated fatty acids that have several effects on the cell, from modifying the physicochemical properties of membranes to the regulation of gene expression. The precise mechanisms of their alleged beneficial effects still are unknown, but their use for preserving human's health are increasing alarmingly. Currently, the use of omega-3 fatty acids is recommended in several diseases, including the diabetes. That is why it is necessary to find out the mechanisms behind their potential beneficial effects. In this work, a model of type 2 diabetes was generated by intraperitoneal injection of streptozotocin (STZ) dissolved in citrate buffer pH 4.6, in newborn Wistar rats (male and female). Control animals were injected with buffer alone. Weaning took place at one month-old, and treatment with omega-3 fatty acids started. Blood glucose were measured every week and cholesterol, triglycerides, and Glucose Tolerance Curves (GTC) monthly. Insulin was measured with an ELISA kit from serum at 1, 3 and 6 months-old rats. Membrane fluidity was detected with fluorescent probes and membranes fatty acid composition analyzed by gas chromatography. The results showed that omega-3 fatty acids had beneficial effects on animals with high hyperglycemia, but little or none beneficial effect on control rats and rats with mild hyperglycemia. Insulin in serum increases in diabetic rats, close to normal concentrations, but in control rats it is likely generating a condition of hyperinsulinemia, which is the first step for insulin resistance and dangerously to development of diabetes. At the beginning of the pathological condition, plasmatic and mitochondrial membrane fluidity increases, but after some time under hyperglycemia, membrane fluidity decreases. Besides, we found that the development of diabetes is different in males and females rats.

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# Combination treatment with ATRA and Fulvestrant inhibits mammary carcinoma cell migration and metastasis in a chicken embryo model.

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It has been reported that approximately 75% of breast tumors are estrogen receptor (ER) positive, and consequently their growth is stimulated by estrogens; current evidence suggests that antiestrogen-blockage of ER is associated either with a small induction or reduction of invasiveness of human breast cancer cells in vitro. All-trans-Retinoic Acid (ATRA), a ligand for retinoic acid receptor, represses breast cancer cell invasion and metastasis, the main causes of death in breast cancer patients. Since ATRA and the pure antiestrogen Fulvestrant (Fulv; Faslodex; ICI 182,780) are individual agents that possess anti-tumor activity in the breast cancer, combination of these drugs may translate into improved therapy for breast cancer patients. In this study we report synergistic inhibition of human breast cancer cell migration and metastasis for combinations of these drugs, the latter using a chicken embryo model.

The effects of different concentrations of ATRA ($10^{-9}$M to $10^{-6}$M) combined with Fulv ($10^{-9}$M to $10^{-7}$M) were examined in the human breast cancer cell line MCF-7 that was stably transfected with the gene that encodes the Green Fluorescent Protein (MCF-7/GFP; Cell Biolabs, Inc). Wound-healing assays were carried out in order to measure ATRA and/or Fulv ability to suppress MCF-7/GFP cell migration. In this assay, Cytosine β-d-arabinofuranoside (Ara-C; 10 μg/mL) was incubated for 2 h prior to the scratch assay, which inhibited DNA synthesis and allowed us to distinguish migration from proliferation. Compared with single-agent treatment, ATRA ($10^{-9}$M) and Fulv ($10^{-9}$M) synergistically inhibited MCF-7/GFP cell migration. Cytotoxic effects were observed only with the combination treatment or in the presence of high concentrations of ATRA ($10^{-6}$M).

We have systematically developed a chick embryo assay to monitor the metastatic properties of breast cancer cells and to study the effect of ATRA and/or Fulv in vivo. MCF-7/GFP cells were inoculated into the allantoic vein of 10-day old chick embryos; six days later embryos were harvested and hearts and livers were processed for laser scanning microscopy identification of fluorescent metastatic foci within these tissues. These results indicate that ATRA and Fulv can function in a combined fashion in regulating MCF-7/GFP cell migration and in vivo metastasis.

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# Effect of matrix stiffness on normal human lung fibroblasts.

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**Background**: Idiopathic pulmonary fibrosis (IPF) is a progressive, irreversible and lethal age-related disease of unknown etiology. It has been proposed that IPF is the result of damage and dysregulation of the alveolar epithelial cells, proliferation of fibroblasts/myofibroblasts and excessive deposition of extracellular matrix (ECM) into the lung parenchyma resulting in matrix stiffness. Consequently, matrix stiffness affects a variety of cell processes including morphology, proliferation, differentiation and migration, and dysregulates TGF-β1 signaling mainly in myofibroblasts. In this context, stiffness of the ECM is considered an important factor, responsible for promoting, perpetuate and amplify the fibrotic response by a positive feedback loop.

**Aim**: To evaluate the effect of matrix stiffness on human lung fibroblasts proliferation and differentiation.

**Results**: Normal human lung fibroblasts were cultures on homemade polydimethylsiloxane (PDMS) matrices of different stiffness (50, 70, 100, 180 kPa) with or without a cover of collagen type I. We obtain Young's moduli of the matrices with uniaxial tension test to evaluate the stiffness of the material. By Western blot assay, we observed that the expression of molecules as alpha smooth muscle actin (α-SMA, a marker of myofibroblasts) was increased according to matrix stiffness on which cells were cultivated. Also matrix stiffness induced an increase in the cell growth rate.

**Conclusions**: The matrix stiffness affects cell proliferation and differentiation of normal human lung fibroblasts.

# Dysregulated calcium signals in tumoral cells MCF-7

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Evidence indicates that the $Ca^{2+}$ content within the sarco/endoplasmic reticulum (ER) plays an important role in the control of cell growth and proliferation by influencing directly the spatial, amplitude and temporal characteristics of intracellular $Ca^{2+}$-signaling. It has been postulated that low levels of $Ca^{2+}$ in the ER lumen is associated with a continuous store-operated $Ca^{2+}$ influx that produces a sustain increase in intracellular $Ca^{2+}$ concentration. This condition makes possible a proliferating stimulus in a variety of cellular systems including cancerous cells. Cells affected with cancer display an unrestrained expansion and some lost in their degree of differentiation. Although cancer is a pathological condition associated to multiple abnormalities, evidence suggests that intracellular $Ca^{2+}$-homeostasis is also profoundly altered. For example, disturbed intracellular $Ca^{2+}$ signaling has been involved in cancerogenesis, induction of cell cycle and inhibition of apoptosis.
One of the principal factors that control the refilling of intracellular $Ca^{2+}$ stores is the sarco/endoplasmic $Ca^{2+}$ ATPase (SERCA) which functions to pump $Ca^{2+}$ from the cytosol into the ER $Ca^{2+}$ store in most cell types. Hence, the aim of this study is to compare in cell lines, one malignant (MCF-7) and the other non-cancerous (MCF-12F) being both derived from human breast epithelium, the next SERCA´s characteristics: 1) Subcellular localization; 2) Capacity to mobilize intracellular $Ca^{2+}$ in response to Tg; 3) Enzymatic activity and 4) Expressed isoforms.

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# Tepary bean lectins fraction *(Phaseolus acutifolius)* induces apoptosis in cell lines of colon cancer.

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## Abstract

New alternatives for therapy are currently seeking against various diseases including colon cancer. Among these alternatives, various compounds of plants are studied as alternative for cancer treatment. Plant lectins are studied in biomedicine for their ability to recognize and bind specifically and reversibly to free or conjugated sugars, such as cell membrane carbohydrates, of particular interest are the alterations of glycocalix of cancer cells. This work focuses on evaluate the mechanism of cell death induction provoked by a Tepary bean lectins fraction (TBLF) in different cell lines of colon cancer. Three cell lines of colon cancer with different genotypic characteristics; HT-29, SW-480 and RKO were used. Cells were maintained under the specific conditions (ATCC®) and dose-response curves were performed to establish the inhibitory concentration ($IC_{50}$) and the lethal concentration ($LC_{50}$). The cells were maintained up to 70% of confluency, fetal bovine serum was reduced from 10 to 2% the amount of for 24h in order to synchronize the cell cycle. The treatments were applied for 8h, the cells were detached by trypsinization (PBS 0.25% trypsin, 0.05% EDTA) and washed with PBS-EDTA (0.05 mM) to avoid agglutination. Flow cytometry assay was performed using Annexin V (Anexin V & cell death, Muse® Millipore) and multicaspases (Multicaspase assay, Muse® Millipore) to confirm the apoptotic effect and lactate dehydrogenase (LDH-Cytotoxicity Assay Biovision®) to discard a necrotic effect in HT-29 cells. The results indicated significant differences in the $IC_{50}$ and $LC_{50}$ between cell lines and between control and treatment groups of each cell line. The Annexin V test shows that the three cell lines were about 30 to 40% apoptosis, but differences between early and late stage apoptosis according to cell lineage were observed. The multicaspasas assay for HT-29 showed an increase of 50% of caspase activity and 50% of cell death which is related to annexin V assay on the same cell line; no significant differences between negative and treated cells were observed for the LDH assay. The results indicate that TBLF induces cell death by apoptosis in the three cell lines; this process is dependent on the activity of caspases in a differential way on the cell lines evaluated, maybe due to their intrinsic genetic alterations.

**Key words**: apoptosis, colon cancer, lectins, Tepary bean

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# *Stevia rebaudiana Bertoni* promotes insulin expression and release in Wistar rats fed with a hypercalorie diet

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**Introduction:** In Mexico, two of the main health problems are overweight and obesity, which in recent years has shown an increase in its prevalence, this in part due to the consume of hypercaloric products and a sedentary lifestyle. A number of individuals with overweight show dysglycemia, hyperinsulinemia, and dyslipidemia, conditions that are characteristic of metabolic syndrome (MS), and constituting an additional risk to the development of diabetes mellitus type 2. WHO has been proposed a general strategy to fight metabolic disorders, and one of the points is change sugar by non-caloric sweeteners, as Stevia rebaudiana Bertoni (SrB). Actually, this plant is used like sugar alternative, and in the treatment and prevention of MS, because has shown hypoglycemic and antioxidant properties, therefore, regulates the metabolism of carbohydrate and lipids. **Objective:** The objective of this work was to investigated the effect of a SrB extract as insulinotropic in rats with MS, which was developed by a hypercaloric diet consumption. **Methodology:** Four groups of Wistar rats were conformed as follows: normocaloric diet (n=10), hypercaloric diet (n=10), normocaloric diet + extract of SrB (i.p. 1g/Kg weight/day; n=10) and hypercaloric diet + extract of SrB (i.p. 1g/Kg weight/day; n=10). These conditions were maintained by 60 days. In each animal was determined: oral glucose tolerance test, insulin response, triglycerides, cholesterol and its fractions (LDL, VLDL and HDL) and free fatty acids. Pancreas homogenates were used for the determination of insulin levels. Additionally, pancreas sections were analyzed with hematoxylin/eosin staining, immunohistochemistry to insulin and PDX-1 expression and immunofluorescence for Chromogranin A. **Results:** After 60 days of hypercaloric feeding, rats developed MS. However, groups administered with SrB extract, showed decreases in serum glucose levels at the expense of hyperinsulinemia. Lipid profile was improved, but decreased HDL and total cholesterol. LDL-C, and VLDL-C showed increases. Meanwhile, in pancreas was observed an increase of insulin levels in all problem groups. Histological analysis showed an increase in the cell number and size per islet; the densitometric analysis for the expression of insulin and PDX-1 showed increases for both, which were according with serum results. However, Chromogranin A levels did not show differences in relation to control. **Conclusion:** Our results let us conclude that SrB extract has insulinotropic effect, which stimulates hypertrophy and hyperplasia in islet pancreas, as well as, a high insulin production associated to stimulus of PDX. Despite the fact that SrB has effects hypoglycemic and hypolipemic effects, the consumption of this plant could produce a pancreatic exhaustion at long-term. This hypothesis requires further studies.

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# Link between triglycerides, SCFAs levels and Colon microbiota functional metabolic profile in the Mexican Childhood Obesity

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**Background**
Obesity is an important public health problem in both developed and developing countries. Colon microbiota provides an additional energy supply through fermentation of undigested carbohydrates, in the form of Short Chain Fatty Acids (Acetic, Propionic and Butyric Acids) (SCFA). Dysbiosis of the distal colon microbial community in Mexican children affects the nutrient absorption, and might contribute to the development of obesity.
**Method**
The microbial diversity profile of normal-weight (n=81), overweight (n=29), and obese Mexican children (n=80) (age 9–11 years) was determined using high throughput sequencing of bacterial 16S rDNA V3- fingerprint libraries from feces. 16S rDNA analysis was done using QIIME pipeline. Prediction of functional profiling was done using PICRUSt.
**Results**
Four phyla were identified: Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. We found three genera increased in overweight and obese, *Faecalibaterium* sp, Lachnospiraceae, and *Roseburia* sp. In contrast, *Succinivibrio* sp, *Erwinia* sp and *Oscillospira* sp genera were significantly reduced in overweight and obese. On the other hand, the genera *Blautia* sp, *Coprococcus* sp, and Enterobacteriaceae sp were significantly increased in overweight phenotype. Fatty acid biosynthesis and Lipid biosynthesis functional genes were predicted to be significantly higher in obese distal colon microbiota. The biochemical studies revealed the bloodstream triglycerides levels were significantly elevated in overweight and obese children (p=0.0001).
**Conclusion**
In this study, we conclude that a particular distal colon microbiota imbalance affects the metabolism of undigested food in Mexican overweight and obese children. We found higher abundance of some bacteria in obese and overweight children, with a higher capacity to harvest energy, which have more abundance of functional genes for fatty acid and lipid biosynthesis pathways. Work financed by Cinvestav-IPN; Fundación Miguel Alemán A. C, CONACyT 163235 INFR-2011-01, and FONSEC SS/IMSS/ISSSTE-CONACYT-233361.

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# Nutraceutical effects by peptides and metabolites from chia: the golden crop of the 21st century

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Chia is an annual plant of the Lamminae family which grows in semiarid climates[1]. Nowadays, chia popularity has been increasing due to its several nutraceutical compounds such as polyunsaturated fatty acids, phenolic compounds and bioactive peptides encrypted in proteins[1,2,3]; these features may provide effective outstanding benefits for the human health. The main protein fraction in chia seeds corresponds to globulins, followed by albumins, glutelins and prolamins[2,3]. Many studies have focused on the isolation, purification and digestion of protein fractions to obtain peptides with biological functions against important diseases in human health and to corroborate them using peptide sequentiation. Angiotensin I-converting enzyme (ACE) is a peptidylpeptidase hydrolase that plays an important physiological role in the regulation of the blood pressure and control of the hypertension; interestingly, treatments are based on the inhibition this enzyme. Proteins of chia seeds are well-known precursors of a range of biologically active peptides which have been been used by other researchers to block the activity of ACE[4]. Phenolic compounds from chia seeds, possess excellent antimicrobial activities as well as antioxidant capacity and some are used against a number of pathological diseases such as cancer, atherosclerosis and brain dysfunctions[5]. The aim of this research was to fractionate protein fractions from different chia cultivars and evaluate the inhibitory activity of peptides against ACE *in vitro*, and the variation of phenolic phytochemical compounds from different cultivars when they grow in different geographical areas. As expected, protein fractions revealed differences in SDS-PAGE, but surprisingly were similar between cultivars. Peptides confronted against ACE enzyme showed that globulin and albumin peptides inhibit in a similar manner the ACE; and these peptides function was corroborated by peptide sequentiation. Phenolic phytochemical compounds revealed differences between chia cultivars, and also in their protocatecuic, rosmarinic, galic and chlorogenic acids contents. These results show that peptides encrypted in chia protein fractions have different inhibitory activity against ACE and could be good alternatives for the hypertension treatment; additionally, metabolites may be considered as functional ingredients with a high antioxidant potential in foods with remarkable benefits for human health.

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# Anti-mitochondrial therapy against triple negative breast cancer.

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**Background:** Triple negative breast cancer is a cancer subtype lacking specific therapy [1]. In order to establish a targeted therapies, the energy metabolism of the triple negative MDA-MB-231 (231) and MDA-MB-468 (468) cell lines was analyzed under both normoxia (21% $O_2$) and hypoxia (0.1% $O_2$) by determining: (1) protein content of glycolytic and mitochondrial enzymes; (2) glycolytic and oxidative phosphorylation (OxPhos) fluxes; and (3) the cell growth sensitivity to glycolytic and mitochondrial inhibitors, and canonical anti-cancer drugs.

**Results:** In both cancer cell lines 24 h hypoxia significantly increased the glycolytic transcriptional key master HIF1-α (99%), which correlated with a significant increment (2-5 times) in GLUT-1, HKI and II and LDH as well as in the glycolytic flux (30-80%). For OxPhos, hypoxia decreased (20%-60%) the contents of COX-IV, 2OGDH, ND1 and ATP-synthase as well as OxPhos flux (75%). Under normoxia, both cancer cell lines mainly depended on OxPhos (75-60%); whereas in hypoxia, both cell lines depended on glycolysis (70-76%). Under normoxia, anti-mitochondrial drugs (casiopeina II-gly and vitamin E-phosphonium derivatives) decreased cancer cell proliferation ($IC_{50}$ values ≈ 2.5 μM) as well as cell migration. These data support the use of mitochondrial inhibitors for the treatment of TN breast carcinoma.

The present work was partially supported by CONACyT-México No. 239930 and 107183 grants.

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# Nicorandil increases activity of complex I and III of the electron transport chain in atrophied muscle mitochondria

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Muscle atrophy is defined as a decrease muscle tissue size due to cellular shrinkage caused by loss of organelles, cytoplasm and proteins. The main characteristic of this pathology is the increase in protein degradation and a decrease in protein re-synthesis. It has been documented that the mitochondrial ATP sensitive potassium channel (mito$K_{ATP}$), is involved in the resistance of the muscle fatigue and recovery from ischemia-reperfusion injury and protection during atrophy process; Nicorandil has been described as a selective mito$K_{ATP}$ opener which confers protection to muscle during stressful conditions, the aim of this study was to determine the protective effect of Nicorandil over the damage caused by atrophy.

C57BL/6 male mice 14-16 weeks-old were used for the experimental basis; these animals were separated into 4 groups: 1) Control, 2) Nicorandil, 3) Atrophy and 4) Atrophy treated with Nicorandil. Atrophy was induced by hindlimb unloading by the tail method. Nicorandil was purveying in 40 mg/kg by intramuscular injection during 14 days. Once finished the treatment, the animals were sacrificed by cervical dislocation and the hindlimb were used for mitochondria isolation, once obtained, the activity of the complexes was measured.

Each complex activity was determined by absorbance changes recorded in a Perkin Elmer Lambda 18UV/VIS in 340nm (complex I), 600nm (complex III). The reaction mixture for measurements contained $KH_2PO_4$ (50mM), succinate (10mM), antimycin A (0.09 µM), KCN (1mM), NADH (100 mM) and Cytochrome c. The activity of each complex was calculated from the slopes of the absorbances graphs using molar extinction coefficient specific for each complex.

Result show an increment in complex I and III activity in atrophy with nicorandil group. These results suggest than nicorandil reduces production in reactive oxygen species protecting muscle from atrophy damage.

Keywords: Muscle, atrophy, ATP sensitive potassium channels, Nicorandil

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# A novel panel of breast cancer-associated auto-antibodies in serum against recombinant tumor-associated antigens.

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**Background**. Recent studies indicate that serum from cancer patients contains auto-antibodies against oncoproteins so called tumor-associated antigens (TAAs), which represent promising diagnostic and prognostic biomarkers. In this study we searched for breast cancer-associated auto-antibodies against individual TAAs. Also we evaluated if multiple TTAs would improve the detection of auto-antibodies. We screened 12 proteins including CEA, CCBN1, c-Myc, p53, Ki-67, Nm23, PRDX6, eIF5A, **PARK7**, GLIO-1, Hsp27 and Hsp70 previously detected as overexpressed in breast tumors of Mexican women's.
**Results**. Enzyme-linked immunosorbent assay (ELISA) was used to detect autoantibodies in 104 sera from breast cancer patients and 50 sera from healthy individuals. Our data showed that antibodies frequency to any individual TAA was low and ranged from 0.96% to 4.8%. Interestingly the successive addition of multiple TAAs represented by panels of three-to-five tumor antigens, resulted in a dramatically increase of positive ELISA reactions. The first panel of three combined TAAs (p53/PRDX6/CEA) had a sensitivity of 19%, while a second set of four TAAs (p53/PRDX6/c-Myc/Hsp70) reached 28% sensitivity. Remarkably, the third panel of five antigens (p53/PRDX6/ c-Myc/Hsp70 /Nm23) showed 24% sensitivity.
**Conclusions**. Our data showed that detection of individual auto-antibodies against TAAs in this particular cohort of patients was low, but will be enhanced by adding multiple TAAs, suggesting that this panel could be useful in Immunodetection of breast cancer. Comparative frequencies of auto-antibodies found in this study with other reports, support the notion that detection could be impacted by geographical and genetic factors of breast cancer patients.
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# Impact of type 1 diabetes on liver Acetyl CoA carboxylase of mice with different biotin *Status*

Cecilia Palmas Bustamante, Laura P. Guerrero Carrillo, Ana L. Vázquez Jiménez, Miguel Anastacio Villanueva, Cecilia Ayala Zambrano, Jorge Omar García Rebollar, Georgina Díaz Herrera, Nancy Y. Mora Pérez, Armida Báez Saldaña*.

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Diabetes Type 1 (DT1) is caused by an autoimmune reaction that causes destruction of β cells of the pancreas, leading to a deficiency in insulin production. Insulin deficiency prevents the regulation of blood glucose levels and also decreases lipogenesis. The fatty acid biosynthesis starts with the synthesis of malonyl-CoA by the biotinylated enzyme; acetyl coenzyme A carboxylase (ACC). Malonyl-CoA is the primary substrate for fatty acid synthesis. However, no studies assessing the activity of the ACC in the DT1 state, so in this study the activity of hepatic ACC of mice induced to DT1 and fed with different biotin content diets were analyzed.

BALBc/AnN, 3-week-old male mice were used to carry out the experiments. Mice were divided into three experimental groups with the same average body weight and dispersion. Each group was fed 1 of 3 alternative diets. Mice of the biotin-deficient (BD) group were fed with a diet lacking biotin; (0 mg of biotin / kg); mice of the sufficient group and supplemented group, with diet biotin content of 1.76 mg of biotin / kg and 97.7 mg of biotin / kg, respectively. During week 5 of experimentation, 6 mice of each group were treated with 5 ip. subdiabetogenic doses of streptozotocin (STZ) dissolved in citrate solution. The other 6 mice of each group were treated with 5 ip. citrate solution. 4 weeks after STZ administration, the mice were sacrificed and the liver was extracted for analysis of the specific activity of ACC.

During the 9 weeks of experimentation and 3 times weekly the average amount of drinking water and food consumed relative to average body weight were calculated. The blood glucose concentration and body weight of mice of the 3 experimental groups were individually recorded every week. The specific activity of ACC was determined by a radio enzymatic method.

STZ-treated mice were classified as DT1 when the fasting plasma glucose level was significantly higher than in the mice of the placebo group. It was found that water consumption relative to average body eight by mice administered with STZ increased significantly in the 3 groups.

In accordance with previous results the activity of ACC was lower in the liver of biotin deficient mice when compared to sufficient and supplemented mice. Our results showed that the average activity of ACC hepatic enzyme did not differ in placebo and DT1 mice.

Then, the mechanism by which biotin is inversely associated with hyperlipidemia in both humans and experimental animals, are not due to alterations in the activity of hepatic ACC.

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# Effects of Pharmacological Concentration of Biotin on Testis and Expression levels of Acrogranin in Mice

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Biotin is a water-soluble vitamin that acts as a carboxylase prosthetic group. Unrelated to this role, biotin at pharmacological concentrations modifies gene expression and biological functions, such as lipid and glucose homeostasis, immune system and development. Studies by our group and others have found that biotin modify reproductive function. In sows, biotin increased the annual productivity, returned the sows to estrus sooner, and resulted in earlier pregnancy. Other studies indicated that biotin supplementation during gestation and lactation increased the number of pigs. In cows biotin positively affected milk production. We demonstrated that biotin supplementation in the diet increased serum estradiol concentrations and modified the ovary histology decreasing the number of primary and Graafian follicles. Little information exist on the effect of biotin on the male reproductive tract. In this study, we investigated the effect of pharmacological concentration of biotin on testis morphology and on the expression of acrogranin a damage marker. Two groups of male mice were fed during 8 weeks post-weaning with different diets: a biotin-sufficient diet containing 1.76 mg biotin/kg diet, or a biotin-supplemented diet (97.7 mg of biotin/kg diet). After 8 weeks of ad libitum feeding mice were euthanized, and blood and testis were obtained. Testis were removed; one of them was placed in liquid nitrogen and stored at -70 C until utilization. The other was fixed in formalin solution. Five-micron histological sections were prepared and stained with hematoxylin & eosin for light microscopy histological or for inmunohistochemical analysis. Our results found that biotin supplementation in the diet induced histological changes in the seminiferous tubules. The major changes were cell disorganization, vacuolization and sperm absence. Inmunohistochemical analysis revealed the absence of acrogranin in the control group, in contrast, acrogranin protein localization was detectable in biotin supplemented mice testis mainly at the nucleus. Western blot showed that biotin-supplemented mice increased the abundance of acrogranin protein compared with the control group. Our findings provide, for the first time insight of the effect of pharmacological concentrations of biotin on male reproductive tract. These results indicate that pharmacological concentrations of biotin might have adverse affects on mice reproduction.

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# TIGAR knockdown carry out to metabolic changes in cancer cell lines affecting survival

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***Abstract***

TIGAR (TP53 induced glycolysis and apoptosis regulator) shows similarity to the bisphosphatase domain of the bifunctional PFK-2/FBPase-2 enzyme. TIGAR functions to lower fructose-2,6-bisphosphate levels in cells, resulting in an inhibition of glycolysis, and an overall decrease in intracellular reactive oxygen species (ROS) levels, correlating with a decreased sensitivity to cell death. Expression of TIGAR may therefore modulate the response to p53 inducing signals, allowing survival in the face of stress signals and might be reversed or repaired. The decrease of the intracellular ROS levels in response to TIGAR may also play a role in the ability of p53 to protect from the accumulation of genomic lesions. Currently, little is known about TIGAR and its knockdown regulation in different cancer types. We hypothesize if TIGAR is knockdown this could be altered the growth of cancer cells and improve response in front to chemotherapy and/or radiotherapy. We silenced TIGAR in several cancer cell lines, using a specific TIGAR siRNAs combination. Different metabolic parameters were assayed. Cell growth rate was evaluated by CV staining. Inmunohistochemistry was used to study cell repair mechanisms in control and TIGAR silenced cells. The cells were subjected to dose-response irradiation and different drugs and surviving were determined. TIGAR silenced shows morphological changes compatible with the lost capacity to form colonies after TIGAR silenced plus radiotherapy and/or chemotherapy exposure.

# Evaluation of the DNA integrity in isolated nuclei obtained from mouse spermatozoa.

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Infertility is a health problem that is increasing every year, where one in six couples is affected. The problem of fertility will be caused by the male in 50% of cases. Currently, the conventional assays that analyses the fertility in males is the spermatobioscopy; however, it has been found that this is not sufficient to ensure fertilization, implantation and a proper embryonic development. Moreover, several studies have shown that the DNA integrity of sperm is important for obtaining a successful product. There are some assays, as COMET, TUNEL, SCDA, that allows analyze the DNA integrity, however, none of them has a wide availability due to its high cost for the need of sophisticated equipment and reagents, in addition to complex methodologies that require longer timer. For these reasons, in our lab we developed a new assay to determine the percentage of unfragmented DNA sperm in a semen sample. Briefly, sperms obtained from mice were incubated in Dithiotreithol (DTT) and Cetyl trimethylammonium bromide (CTAB ) solution to eliminate the cytoplasmic and nuclear membranes to obtain isolated nuclei and disintegrate sperms with fragmented DNA. In the other hand, an aliquot was incubated at 39ºC overnight to induce DNA fragmentation. Later, the aliquot was incubated in DTT/CTAB solutions to eliminate sperm membranes. To evaluate the DNA integrity in both samples, COMET and TUNEL assay were performed. The number of isolated nuclei from sperms after incubation in DTT/CTAB diminish and did not show fragmentation in its DNA. The percentage of cells eliminated was similar to the number of cells with fragmented DNA before incubation in DTT/CTAB. We can conclude that this method could be used as a new methodology to evaluate cells with fragmented DNA in a semen sample. Moreover, this method becoming more precise, at lower cost and made in less time.

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# Diabetes Mellitus type 2 decrease migratory capacity and store operated calcium entry in cardiac fibroblasts

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**Introduction**. Cardiac fibroblasts (FiC`s) are most abundant cells in the heart. They form the extracellular matrix and participate in the electric and metabolic cross-talk with cardiomyocytes. Calcium ion is an ubiquous second messenger, its concentration is highly regulated in the cytoplasm since it is involved in many functions including the regulation of cellular migration. Incidence of *Diabetes mellitus* (DM) in Mexico will increase from 9.7% to 15.2% in 2035. Cardiac fibrosis and stiffness, two processes involving FiC's, are some usual complications of diabetic patients.
**Aim**. In this work we used an animal model of DM type 2 to study the changes in calcium signaling, the migratory capacity of FiC's and their possible interplay.
**Methods**. We isolated and cultured cardiac fibroblast for 13 weeks old male Long Evans (LE) rats or from Obese Zucker Diabetic Rats (oZDF). Fibroblasts from passage 1 were used to measure intracellular calcium using FURA-2AM and to quantify migration through the wound-healing assay in the presence of 2mM $NiCl_2$, 10µM BTP-2 or condition media from oZDF or LE rat adipocytes.
**Results**. We found in cardiac fibroblasts derived from oZDF rats a decrease of 80% in migration distance ($p<0.05$); this effect was abolish when cells were incubated in condition media from Long Evans rat adipocytes. According to the observed effect, when we blocked the store operated channels, the migration was reduced to 54% of control condition while inhibition of NCX activity by $NiCl_2$ reduced migration to 22% of control. Both, SOCE and NCX are key mechanisms in the calcium clearance on fibroblasts. On the other hand, the migration was not affected when blocking refilling of calcium stores by the SERCA inhibitor, ciclopiazonic acid. These data is in accordance with the results obtained by calcium fluorimetry: oZDF fibroblast presented a 16% reduction in the calcium release from endoplasmic reticulum if compared to Long Evans fibroblast but a reduction of 51% in the store operated calcium entry.
**Conclusion**. Calcium entrance decrease in oZDF FiC's and the effect of blocking store operated channels using BTP-2 suggest a central role of these channels in the migration of cardiac fibroblasts. It is important to highlight that the observed effect is not due to smaller calcium stores but could be explained by a decrease in the functional expression of SOC channels.

# Isolation of an RNA aptamer population against HPV-16 L1 protein.

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In recent years, production and purification of the Human Papillomavirus (HPV) L1 major capsid protein has acquired especial relevance in scientific and commercial contexts due to its intrinsic property of self-assemble into virus-like particles (VLPs), which have been exploited to develop vaccines against different HPV types. In addition, recent reports have described two novel inhibitors against L1 monomers that preclude capsid assembly, suggesting a new series of prophylactic and therapeutic agents against HPV.

Aptamers are synthetic single-stranded oligonucleotides, that bind tightly and specifically to target molecules; most aptamers are selected by SELEX, an iterative process that mimics evolution to obtain the best ligands through an *in-vitro* selection process. During the last two decades, a plethora of aptamers have been isolated versus a wide range of targets, such as ions, proteins, virus, and eukaryotic cells; revealing new exciting properties beyond the canonical functions of nucleic acids.

The aim of the present work was to isolate an aptamer population against the L1 monomeric protein of HPV-16. Thus, five SELEX rounds were performed using a randomized RNA pool with 430 variants and the GST-L1 fusion protein as target protein; each round was divided in two steps of positive selection and one step of negative selection. Binding analysis were performed by Slot Blot assays, proving unspecific interaction between the RNA isolated from the last round and the protein GST-L1. Consequently, is mandatory to improve SELEX astringency to increase the probability of isolate aptamers against the L1 protein.

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# Serum iron is associated with dyslipidemia in a population of Mexico City

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**Introduction:** Dyslipidemia is a relevant cardiovascular risk factor with an increasing prevalence in Mexico. Several studies in animals and a few in humans suggest that trace metals could contribute to modify lipid status. Hemochromatosis is an iron overload disease, in which altered lipid profile is observed. Iron enriched diets in animals have led to contradictory results, since both high and low cholesterol have been found.
**Objective:** The aim of this work was to evaluate the association between different types of dyslipidemia and serum iron in participants enrolled in the Tlalpan2020 cohort.
**Methods:** Tlalpan2020 is an ongoing cohort study, whose main objective is to estimate the incidence of hypertension in Mexico City population. Participants are clinically healthy people aged between 20 and 50. Exclusion criteria are hypertension, diabetes, dysthyroidism, cardiovascular or renal diseases, and pregnant women. Six hundred and thirteen participants of the cohort were included in this sub-study and were classified in six groups according to the Adult Treatment Panel III criteria: without dyslipidemia (WD), isolated hypertriglyceridemia (HT), isolated hypercholesterolemia (HC), hypoalphalipoproteinemia (hA), mixed-dyslipidemia (MD) and low HDL cholesterol-high LDL cholesterol (LH-HH). Serum iron was measured in an automatic analyzer. Analysis of variance was performed, considering dyslipidemia type and sex as fixed factors, and age as a covariate. Partial correlations between serum iron and parameters of lipid profile were also performed, adjusting by age and sex.
**Results:** Mean age of participants was 35.9 $\pm$ 10.1 years old, 64% of them were women; types of dyslipidemia were distributed as follows: 30% WD, 32% MD, 17% hA, 16% HC, 3% HT, and 2% LH-HH. Serum iron behaved differently depending on sex and type of dyslipidemia, being lower in the hA group and higher in the HT group; significant differences were observed between the hA group an all the others, except for the LH-HH group. Iron positively correlated with HDL-cholesterol ($r=0.147$, $p<0.001$) and negatively with triglycerides ($r=-0.087$, $p=0.032$); additionally, iron negatively correlated with the lipid accumulation product ($r=-0.122$, $p=0.003$).
**Conclusions:** We found an association between dyslipidemia and serum iron; such association seems to be given by the relationship of iron with triglycerides and HDL-cholesterol. We cannot determine whether iron alters the lipid profile or the lipid status affects iron levels; we will possibly get more information to this respect after completion of the follow-up of this cohort.
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# Synergistic evaluation of anti-mitochondrial and clinical drugs in cervix cancer growth

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**Background:** The clinical or canonical drugs used against cervix cancer results in low patient's quality life and cancer regression. Therefore, alternative therapies should be considered to deter cervix cancer progression. Previous studies showed that cancer cells are highly susceptible to Casiopeina II-gly (Cas II gly) a lipophilic copper-based drug due its high mitochondrial ATP dependence. Although Cas-IIgly affects tumor oxidative phosphorylation, also compromises heart function. In order to diminish the Cas II-gly toxic effect, sub-$IC_{50}$ doses of the drug were combined with well-known antineoplastic drugs to attain a synergistic effect [1].

**Results:** The effect of 16 clinical drugs and Cas-IIgly on cervix HeLa proliferation was determined after 24 h of culture in both monotherapy and in combination with Cas-IIgly. The monotherpay $IC_{50}$ values for clinical drugs were in the milimolar range (0.1-10 mM), except for paclitaxel (30 μM); whereas for Cas-IIgly the $IC_{50}$ value was 1.5 μM. Synergism identification was determined by using the additivism model reported by [1]. From all clinical drugs assayed only 5 (cisplatin, gemcitabine, cyclophosphamide, carboplatin and paclitaxel) showed a supra-additive synergism. After combination of CasII-gly with clinical drugs at sub$IC_{50}$ values, the $IC_{50}$ of CasIIgly was significantly diminished by 40-50%. It should be noted that Cas-IIgly or clinical antineoplastic drugs at all doses assayed did not affect the growth of 3T3 mouse fibroblast. These results clearly indicate that paclitaxel, cisplatin, gemcitabine, cyclophosphamide and carboplatin increase Cas-IIgly potency which may be used as a potential combinatory therapy against cervix cancer without apparent effect on non-cancer cells.

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# Biomarker profile of oncogenic proteins in patients with cardiac myxoma

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**Background.** Atrial myxoma is the most common hereditary primary cardiac tumor considered as of benign-type [1] but associated with cardiac failure. However after surgical removal, high recurrence is observed which in turn promotes cardiac failure associated with patient poor quality life. Although myxomas may be easily detected by echocardiography, there are not potential biomarkers for malignancy progression. In order to characterize cardiac myxoma´s malignancy profile a proteomic study was performed analyzing the contents of well-established oncogenes (C-Myc, KRAS and HRAS) and epithelial mesenchymal transition proteins (fibronectin, Vimentin, β-catenin, Snail and MMP9) in cardiac myxomas and compared to their adjacent cardiac tissue and normal heart tissue. Statistical analysis between experimental groups was performed by using ANOVA and ROC curves for non impaired samples [2].

**Results.** Comparison of myxomas vs. adjacent tissue revealed great similitude in the contents of C-Myc, KRAS, HRAS and HIF1-α, indicating that adjacent tissue express oncogenes and proteins related with malignancy phenotype. However, other proteins such as p53 + fibronectin + vimentin + β-catenin increased in myxomas vs. adjacent
t tissue. Strict statistical analysis with ROC curve revealed that these proteins may be considered as good cancer biomarker because they showed area under the curve value of ≥ 0.9 [2].
Compared to health heart, cardiac myxoma showed a significant increment (15-90 times) in the oncogene C-MYC and in the transcriptional factors HIF1-α and p53 reveling a possible biomarker panel for malignancy detection in cardiac myxomas.

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# Differential effect of molecular iodine in mammosphere culture of breast cancer MCF-7 cells

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Mammary carcinoma is the malignant tumor with highest incidence worldwide and the first cause of death in women in reproductive age. It is estimated that 90% of mammary carcinomas have the potential to generate metastasis. In recent years several studies have focused on improving the conventional chemotherapeutic treatment by using natural molecules to limit chemoresistance and avoid significant increases in toxicity. Molecular iodine ($I_2$) is a chemical form of iodine that exerts significant antineoplastic effects on several cancer cells, and whose actions could be mediated by the induction of differentiation mechanisms. There are many theories to explain the way carcinogenesis arises and progresses. Among them, cancer stem cell (CSC) theory proposes that only the stem cells present in the mature tissue, after converted into carcinogenic cells, possess tumor-initiating properties and metastatic potential. Putative CSC have been described and characterized in breast cancer, where the $CD44^+/CD24^-$ surface marker profile has been considered a canonical CSC characteristic. Moreover, in vitro mammary cancer MCF-7 cells cultured under serum-free, non-adherent conditions lead the selection of highly enriched CSC by the formation of spherical cell clusters called MCF-7 mammosphere (MCF-7/M) with high invasive capacity. On the present work we used this approach to evaluate the effects of $I_2$ in cell proliferation and mammosphere formation. Results showed that after 72 hours 90% of the MCF-7/M culture adopt a mammosphere pattern and exhibit high expression of CD44 marker. Iodine supplementation is accompanied by decrease in cell proliferation in CSC and parental cancer cells; it also impairs mammosphere formation in a dose-response manner. Cytometric analysis showed that MCF-7/M culture exhibited a dominant $CD44^+/CD24^+$ sub-population and $I_2$ supplement exerted a differential selection through the phenotype $CD44^-/CD24^-$. All these results suggest that $I_2$ maintains its antiproliferative effects in CSC and parental cancer cells by forcing their differentiation into a less invasive phenotype and thereby inhibiting their tumorigenic capacities. Studies analyzing this hypothesis are currently ongoing.

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# *In vitro* identification of differential gene expression in patients with colorectal cancer in Baja California Sur

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## ABSTRACT

**Introduction.** Colorectal cancer (CRC), characterized as an uncontrolled cellular growth disease in colon, rectum and appendix, represents the second cause of death for cancer in Mexico. In 2008, there was reported that Baja California Sur had the second highest incidence of patients with CRC nationwide. Parrafin-embeded clinical samples analysis has been used for diagnosis, prevention and therapy of this pathology. Therefore Molecular Biology techniques would be an option to improve the clinical diagnosis. This suggests the development of diagnosis and prognosis techniques based on differential gene expression studies as an alternative that could be applied in this region. The aim of this study is to identify genes with differential expression that would serve as a genetic signature for CRC in Baja California Sur from formalin-fixed paraffin-embedded tissues (FFPE).
**Methodology.** RNA from 30 samples of FFPE colon tissue and CRC tissue of different patients was purified with a home-made standardized protocol that includes a deparaffination with xylene and a guanidine thyocianate and phenol/ chloroform isolation technique. cDNA was synthetized and quantified with spechtrofotometry. Specific primers of endogen genes and 5 differential expressed reported genes were designed with online resources: (a) GNB protein (cellular signaling); (b) Cromogranin A (hormonal regulation), CA2, ($CO_2$ procesing); (d) IITP protein (immune response); and (e) NDK1 (nucleotide metabolism). Quantitative Real Time PCR will be used to study the relative changes in gene expression through statistical analysis with $2^{-\Delta\Delta Ct}$ method.
**Results and conclusions.** Concentrations of purified RNA obtained with the standardized protocol varied from 200 to 1000 ng/µl, varying significantly with respect to sample containing, which are optimal for molecular analysis. 260/280 correlation results were between, 1.6-1.9, which indicates optimal purity compared to molecular studies using FFPE tissues. cDNA synthesis was accomplished, obtaining a yield around 80%.

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# Proteomic study on mitochondrial complex IV disease using primary skin fibroblast cultures.

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Mitochondriopathies are result of mutations affecting the respiratory chain complex and oxidative phosphorylation (OXPHOS) resulting in a loss in ATP synthesis. These diseases have an incidence of 1/5000 to 1/10000 for children and adults respectively, are multisystemic diseases and mainly affect organs with high energy demand as heart, brain and nervous system. The OXPHOS system is integrated by four complexes and the ATP ase, all of them are located at mitocondrial cristae where are found as individual monomers or assembled into supercomplexes or respirsomes which could be affected by structural issues involved in mitochondriopaties.

Mitochondriopaty determination and analysis is performed on a muscle sample which is not enough since complete diagnosis or analysis, mainly in pediatric patients, since different procedures and sample treatmenat are required. One good alternative to overcome this difficulties is the primary skin fibroblast culture since allows an additional source of biological material and the possibility to complete a better set of probes for molecular studies and a full understanding of these diseases. Then the aim of this work is to get primary skin fibroblast cultures from mitochondriophaty patients to evaluate their utility in diagnosis and molecular mechanism of mitochondriopathies.

Then skin biopsies were grown and fibroblast cells were tested for phenotypic conservation since could be lost in cell with less energetic demands. Cell cultures will be analyzed by zimography on native gels and spectrophotometric catalytic assays. Phenotipic profile also will be verified by ATP synthesis assays. Finally confirmed cultures will be analyze by 2D proteomics (IEF/SDS).

We grown six primary cultures confirmed with disease by ATP assays, trhee of them are complex IV defects. Complex IV was confirmed by ATP synthesis assay. These cultures will be grown in order to perform IEF and 2D electrophoresis and to determine the molecular signature or relevant affected proteins in mitochondrial complex IV disease landmark.

The method outlined here could be useful to understand molecular mechanism underlaying mitochondrial complex IV disease and for therapy design.

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# Analysis of circulating microRNAs in plasma of gastric cancer patients

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Gastric cancer (GC) is the fifth most common neoplasia and the third leading cause of deaths worldwide. It is estimated that there will be an increase of 50% in the incidence rate in the next ten years. CG has a multifactorial origin, an important factor is the microRNAs expression deregulation, these are regulatory molecules mRNA expression. MicroRNAs have been evaluated in plasma, and have a specific expression profile per neoplasia. The aim of this study was to determine microRNAs expression levels in patients GC plasma and propose them as probable biomarkers. GC and non-atrophic gastritis (control) plasma were used. Samples from a biobank patients of ≥50 years old, males, without treatment. Total RNA was extracted, and cDNA synthesis was done. microRNAs qRT-PCR was carried out with a commercial array. Expression analysis was performed using the http://pcrdataanalysis.sabiosciences.com/mirna/arrayanalysis.php software. Stability analysis was done to determine the normalizers microRNAs. hsa-miR-18a-5p and hsa-miR-29b-3p were the best combination. We determined two under-expressed microRNAs in GC plasma: hsa-miR-200c-3p and hsa-miR-26b-5p, they were over-expressed in non-atrophic gastritis (control). These microRNAs were related to cancerous processes by *in silico* analysis. We performed KEGG analysis using Diana mirPath software to establish networks in which hsa-miR-200c-3p y hsa-miR-26b-5p might be involved. CyTargetLinker software was used to establish a network to determine target genes of these microRNAs. The relationships found in both analyzes mentioned, showed that hsa-miR-200c-3p participates in pathways and regulates genes linked to the process of epithelial mesenchymal transition, and hsa-miR-26b-5p with cell cycle regulation. These findings are being checked experimentally in our research group. We will do this study to large scale, to determine if hsa-miR-200c-3p and hsa-miR-26b-5p could be potential markers in patients GC plasma.

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# Identification of IgE binding proteins from *Ligustrum lucidum* using an Immunoproteomics approach

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Pollen proteins derived from the Oleaceae family are important cause of allergic respiratory disease some of which include the trees olive, ash and ligustrum. To date more than 30 different allergenic proteins have been identified from ash and olive. The genus Ligustrum contains approximately fifty species, including *Ligustrum lucidum (L)* which grows worldwide. The wide distribution of Ligustrum, as well as the raising cases of respiratory allergy related with these plants, demands better understanding of ligustrum pollen allergens. Recently we have identified 6 IgE-binding proteins using serum from monosensitized allergic patients (BBRC 2015, 468:788). The present work investigates the proteomic profile of the Ligustrum *L.* pollen using sera from Ligustrum polysensitized patients suffering allergic respiratory disease. A modified phenolic extraction method was used for the extraction of pollen proteins which were analyzed by two-dimensional electrophoresis and LC-MS/MS. The 2-DE gels revealed 23 spots which were found to contain 28 different IgE-binding proteins.

These novel IgE reactive components could use to expand the panel of well-defined Ligustrum *L* pollen molecules for a more efficient allergen-based diagnosis and therapy.

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# Effect of biotin supplementation during different stages of pancreatic development

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Pancreatic islet development is critical to maintain glucose homeostasis later in life. In rodents, pancreatic islets full development comprehends embryonic, lactation and the first week post-weaning; in this later, it is accomplished islet maturation which culminates with the capacity to secrete insulin in response to glucose and attain archetypical islet structure. Biotin is a water-soluble vitamin that besides acting as a carboxylase prosthetic group, in pharmacological doses is capable to modify gene expression and diverse biological functions as metabolism, reproduction and embryonic development. In previous studies we demonstrated that eight weeks of a biotin supplemented diet after weaning, modified islet morphology, and mRNA abundance of transcription factors and proteins that are critical in glucose induced-insulin secretion and pancreatic islet development. In the present study we investigated which are the effects of biotin supplementation on the offspring pancreas development and glucose homeostasis when the vitamin is administrated: 1) during critical stages of development (pregnancy and lactation), or 2) during maturation (first week after weaning). In the first model, adult female BALBc/ANN Hsd mice were fed a biotin control, or a biotin supplemented diet (56 times that contained in the control diet) during pregnancy and lactation, at d21 of postnatal life (weaning day) the pancreas of female offspring were evaluated for islet insulin secretion and islet morphology. To investigate the effect of biotin during maturation, at weaning, 21 days old female BALBc/ANN Hsd mice received either the control or biotin supplemented diet over a period of one. The results showed that, compared to the control group, the offspring resulted from mothers that received biotin supplementation during pregnancy and lactation showed no difference on the concentrations of fasting glucose, serum insulin concentrations and static insulin secretion. Morphometric analysis revealed that the biotin supplementation does not modify the average size of the pancreatic islet: the proportions of the glucagon- and insulin positive area or the number of islet per total of pancreas area. In contrast, a biotin supplemented diet administrated during one week after weaning increased the number of islet per total of pancreas area with an augment in the average size of pancreatic islet, and increased insulin positive area by islet. In conclusion, biotin supplementation does not affect early offspring islet development (pregnancy and lactation), however is able to produce changes in the maturation stage (1 week after weaning), suggesting that pharmacological concentrations of biotin affect islet maturation network.

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# "Early differential Immuneregulation in Acute Lung Injury induced by Systemic Inflammatory Response Syndrome or Sepsis"

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## Abstract

**Background**: Systemic Inflammatory Response Syndrome (SIRS) is an exaggerated immune response that affects a body systemically, clinically known as Sepsis when is identified a systemic infection by any biological agent (≈29% mortality). The development of SIRS leads to multiple organ failure which includes the Acute Lung Injury (ALI) with subsequent development of Acute Respiratory Distress Syndrome (ARDS), the leading cause of respiratory failure. There are several studies of different molecules and cells that could have a beneficial or deleterious effect during septic process, however is no clear what happens simultaneously in lung tissue from the point of view immunoregulatory. **Objective**: To analyze the expression of different cells and immunoregulatory molecules that help to understand why? and how? we can prevent acute lung injury during ARDS or Sepsis. **Material and Methods**: Spleen and lung cells from C57BL/6 mice treated with 10, 100, 200 and 400 micrograms of intraperitoneal LPS were used for immunophenotypic markings of subpopulations (CD3+, CD19+, CD14+, CD3- NK1.1+, NK1.1+ CD3+) and combined with other functional markers (CD69, Gal-3, Gal-9) for analysis by flow cytometry, also we obtained supernatants and RNAm from cell cultures for cytokine analysis (IL-1 beta, TNF alpha, IFN gamma, IL-17, IL-10) by ELISA and RT PCR. **Results**: Our results show that B cells are increased in spleen (≈60%) but dropped dramatically in lung (30% vs 8%), both organs maintaining their activated phenotype (CD69 60-80%), macrophages increase marginally in spleen (7% vs. 10%) and significantly in the lung (3% vs 40%), in the meantime the other subpopulations show no increase or not as marked as those already mentioned. Regarding the other markers analyzed, it was found that both cell percentage and molecule numbers of certain markers (determined by the mean fluorescence intensity) are increased significantly, for example the galectin-3 and -9, but the expression of these molecules it is different in some subpopulations of spleen and lung. The determination of cytokines in supernatant showed that TNF alpha, IL-17 and IL-10 are produced by cells of both tissues, with an increase of IL-10 production in lung. **Conclusion**: These results suggest a differential immunoregulation between the cell subpopulations in study which gives them a characteristic molecular expression or secretion, same that can be reversed in other organs such as lung, resulting in a different susceptibility to suffer damage or infection.

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# Cardiovascular risk factors defined by the global scale Framingham in a group of adults residing in Mexico City.

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## Abstract

The conventional risk factors, especially hypertension, hypercholesterolemia, diabetes mellitus and smoking are predictors useful for cardiovascular morbidity and mortality, knowing these aspects can make useful predictions, which at some point may result in reduced events clinical preventive actions.

Prevention capacity by detecting and controlling risk factors, may be limited, because it may have an asymptomatic organ damage. So to find new ways of cardiovascular risk (CVR), using techniques that enable such detection is important in reducing cardiovascular disease. Framingham scale is useful in clinical practice and tables are considered useful in clinical decision making in patients with cardiovascular risk.

**Objetive.** Stratify the risk factors influencing the prognosis of cardiovascular risk through the Framingham scale using point of care (POC) in a group of adults residing in Mexico City.

**Methods**. This was a descriptive and observational study. 100 adults were evaluated: 62 women and 38 men; the age range was 32-60 years. People were chosen in a public charity institution by random and stratified sampling. The following parameters were analyzed: sex, age, diabetic patient, smoking, systolic blood pressure, total cholesterol, and HDL cholesterol. Serum lipids were determined by Point of care test. Cardiovascular risk stratification was performed by Scale Framinham.

**Results**. Of the study population, 70% had low CVR, 18% CVR average and 13% higher CVR. Most women did not have high (78%) cardiovascular risk, while men had a higher risk (84%). The total sample, 8% had diabetes type 2 and 17% hypertension (mostly men). In addition, a significant percentage (35%) was smoking.

**Conclusion**. In this study cardiovascular risk was found in the majority of the population. Is important to consider that the risk factors included by Framingham present in this population are related to the occurrence of other diseases such as metabolic syndrome. So it is important to set goals for control and prevention in this population group, especially in males.

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# Relationship between total cholesterol and triglycerides levels with Body Mass Index and dietary ingest in a group of workers

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## Abstract

**Introduction.** Different studies show that elevated plasma levels of total cholesterol (TC), triglycerides, obesity and unhealthy diet are closely associated with the highest incidence of coronary heart disease (CVD). Cardiovascular diseases are the most important cause of disability and premature death throughout the world and are considered a significant, universal public health problem. It is recognized that CVD impact the productivity of working age adults who are the economic engine of most of the countries.
**Objective:** The aim of the present study was to determine the relationship between total cholesterol and triglycerides levels with Body Mass Index and dietary ingest in a group of workers.
**Methods**. An observational cross sectional study was conducted using data of 29 subjects between 25 and 50 years. All participants were workers of a private company localized in Tláhuac, Mexico City. Blood cholesterol and tryglicérides were measured using standard methods and calibrated tools at the same laboratory. Weight and height was measured using a portable stadiometer and scales SECA®. Dietary ingest was evaluated by a food frequency questionnaire. Anthropometric and dietetic variables were obtained by a trained nutritionist. The chi-square test of independence was used in order to evaluate if there are significant differences in qualitative variables. The SPSS 19.0 software was used for the statistical analysis. All calculation were made at level of significance of $p<0.05$.
**Results.** The 62% of the sample had good cholesterol values (<200mg/dL), the 38 (11 workers) presented high cholesterol values. The 17.3 % of the sample dataset (5 workers) had good triglicerydes values (< 150 mg/dL), while the remaining 82.7% (24 workers) had high triglicerydes values, resulting in a greater risk of cardiovascular diseases.
High BMI ($p<0.05$) and low intake of fruits and vegetables ($p<0.05$) were associated with high cholesterol and triglycerides levels.
**Conclusion.** There was a high prevalence of dislipidemias in this population and it was found that high BMI ($p<0.05$) and low intake of fruits and vegetables ($p=0.02$) were associated with high cholesterol and triglycerides levels in this groups of workers.

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# HIV: discrimination and stance young university people infected

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**Summary**

Acquired immunodeficiency syndrome (AIDS) is a condition that has grown exponentially in recent years as the causative agent of human immunodeficiency virus (HIV). Around 36.9 million people worldwide were living with HIV by 2014 and in Mexico, Tabasco ranked third of the states with the highest rate of new cases diagnosed. We present preliminary results of a sample of 399 new students to campus Chontalpa of the Universidad Juárez Autónoma de Tabasco. Who they responded to a survey that is closed questions to determine the degree of knowledge of the virus and its position on infected persons. The aim of this work is to know how much young people know about the virus and its transmission, what they think about people infected and if they are informed of how to detect the virus in the body. In conclusion there is still a lot of misinformation in society the mode of transmission of the virus and discrimination by society towards people infected with HIV.

**Keywords:** Discrimination, Perception, HIV.

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# Comparative Side Effects Of Two Inhibitors Of The 3-Hydroxy-3-Methylglutaryl Coenzyme-A Reductase: Atorvastatin And Rosuvastatin, When Administered In High Doses To Rodents With A Cholesterol-Rich Diet

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**BACKGROUND.** Statins are the drugs of choice for first and second prevention of cardiovascular disease (CVD) .They were introduced in the clinical practice since 1987. Endo isolated compactin from *Penicillium citrinum* cultures. They are extensively used in patients with high cholesterol level in serum in order to control this CVD risk factor. Recently the American Heart Association and the Endocrine Society recomended an aggresive initial treatment of patients with high CVD risks. As other pharmaceutical compound, statins are not exempted of having undesirable side effects, specially when used in high therapeutic doses, for prolongued periods of time, or in patiens with intolerance to this type of drugs. Animal models can be used to evaluate and compare the side effects of statins. The association of high doses of statins and a cholesterol-rich diet is very harmful for rodents. A previous report included data from some of these compounds. **OBJECTIVE**. This study compared side effects of atorvastatin (ATV) and rosuvastatin (RVT), when they were administered to hypercholesterolemic CD-1 male mice. **METHODS**. The animals received a laboratory chow diet (CD) with 18% protein, 5% fat and 5% fiber. The food was powdered and the statins were added in the amounts described below. Hypercholesterolemic diet (HD) contained 2% cholesterol, 0.6% sodium deoxycholate. **DESIGN**. Experiment 1 (n=6): CD + ATV (0, 50, 100, 200, 400 or 500 mg/Kg/day); HD+ATV (0, 50, 100, 200, 400 or 500 mg/Kg/day). Experiment 2(n=6): CD + RVT (0, 20, 50, 100, 200, 400 mg/Kg/day); HD + RVT (0, 20, 50, 100, 200, 400 mg/Kg/day. Experiment 3 (n=8): CD+RVT (0, 20 mg/Kg/day); HD + RVT (0, 20 mg/Kg/day. In experiments 1 and 2 the percentage of survival was evaluated as well as the morphology of the liver and the hepatocites mitochondrial structure after treatment. In experimente 3, it was evaluated day by day the biochemical parameters in blood, the respiratory function in the hepatocytes mitocondria and also the morphological changes in the liver tissue. **RESULTS.** ATV or RVT coadministered, in high doses, with a cholesterol-rich diet to mice were very harmful and produced precocious death. RVT is a more active drug than ATV but also has more powerful side effects on the liver structure and function. Both statins modify the respiratory function of hepatocytes mitochondria. **CONCLUSION**. ATV and RVT, like other statins, are currently used in the prevention and treatment of CVD, but their potential side effects, even in therapeutic doses, must be considered by physicians and patients.

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# Antioxidant capacity and physicochemical characteristics of wine from jamaica (*Hibiscus sabdariffa*, L.)

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**ABSTRACT**
Nowadays there is a great interest over the consumption of foods and beverages that provide health benefit, i. e. those containing antioxidants from natural sources. Roselle contains an important amount of antioxidants like anthocyanin, polyphenols and ascorbic acid, and is mainly used to elaborate cool beverages, infusions and jams, with a wide market within México. On the other hand among the alcoholic beverages, it has been demonstrated that red wine also presents antioxidants, mainly anthocyanins and polyphenols, which have cardioportective properties; and this is a product with a worldwide market. Therefore, in this investigation, the chemical composition, total phenolic content, vitamin C, total monomeric anthocyanins and antioxidant activity (DPPH) were determined from the Roselle calyces (*Hibiscus sabdariffa,* L.) of "Colima" variety, harvested in Puerta Anzar, Colima, México. An aqueous extact was elaborated, and with it, a jamaica wine was prepared by fermentation with *Saccaromyces cerevisiae*, to which physicochemical, antioxidant and sensorial properties were later analized. Among the main results obtained in the wine, the total anthocyanins value was 22,32 ± 0,32 mg/L cyanidin-3-glucoside equivalents and total phenolics of 60,83 ± 1,69 mg GAE/100 mL. Also, the beverage presented an 86.2% of scavenging activity. The pH of the wine (2.86) remained low after fermentation, which preserved the characteristic red color of Roselle due to the existence of the flavillium cation from the present anthocyanins. In the sensorial analysis, the wine maintained the characteristic Roselle flavor, red color similar to that red wine and alcoholic content of 9 °GL without significant astringency or acidity.
**PALABRAS CLAVE:** *Hibiscus subdarifa* L., total phenolics, anthocyanins, antioxidant activity.
AREA: Medicina, Salud y Nutrición

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# Proteomic analysis of exosomes from serum of breast cancer women and citokines-stimulated cell lines

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Breast cancer prognosis survival is linked to early detection, in this context Extracellular Vesicles (EVs) have emerged as novel potential biomarkers. They are released by cells and then transport a wide variety molecular cargoes such RNAm and proteins, depending on the cell type and the stage of diseases.

Also It has been observed that the molecular exosomal content is modified by environmental stimuli, however, it has not been explored the role of cytokines on the protein pattern of exosomes from stimulated breast cancer cell lines.

To identify potential biomarkers on exosomes, we isolated exosomes from serum of patients with breast cancer stage I and II. To explore if the cargoes are modified compared to exosomes from healthy women. We enriched exosomes using “Total Isolation Exosomes Kit”, the precipitated samples were lysed and analyzed by MALDI-TOFF. We test two samples by mass spectrometry analysis: one was realized with protein solubilized from bands separated by SDS-PGE and the second was performed with soluble proteins in ammonium bicarbonate. In the first analysis 156 proteins were identified: 17.7% have been reported in exosomes, 27.5% are located on extracellular region, 28.5% catalogued in immune response and 13.3% related to transport and cell growth. In the second analysis,190 proteins were identified: 13.3% have been reported on exosomes, 22.8% described in extracellular region, 15% involved in cell growth and 13.5% related to protein metabolism.

However, a lot of serum proteins masked the exosomal proteins,for this reason we chose immunocapture using anti-CD63+ coupled to magnetic beads to purify exosomes population without contamination from other EVs or serum proteins.Then the exosomes obtained were lysed and analyzed by mass spectrometry.

On the other hand, to explore the role of IL-1β cytokine on protein pattern of exosome, we compared the protein content of exosomes from the breast cancer cell lines MDA-MB-231 and MCF7 stimulated with this cytokine for 24 hours. The exosomes of the culture medium were isolated and analyzed by spectrometry mass. Also, to compare Ezrin protein expression, which has been considered as a malignancy indicator in other types of cancer western blot was used. In addition, the presence of CD24, which has been proposed in several studies as breast and ovary marker, was corroborated.

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# Leishmanicidal drug design. Induce fit studies to find potential inhibitors against arginase from *Leishmania Mexicana*.

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**Introduction:** Leishmaniasis is a parasitic disease that is transmitted to the host by the phlebotomine sandfly bite, this disease can involve the skin, mucous membranes and viscera. Drug therapy for leishmaniasis is inadequate due to the severity of side effects, toxicity, high cost, and the emerging drug resistance. Therefore it is important to looking for new drugs against leishmaniasis. Polyamines are molecules important for parasite survival and proliferation. Arginase, is the first enzyme in polyamine biosynthesis and catalyzes the hydrolysis of L-arginine to L-ornithine and urea. Here we used this enzyme as starting point to search molecules with potential leishmanicidal activity.

**Methods:** Induce fit studies were made using the crystal structure of arginase from *Leishmania mexicana* (LmARG, PDB: 4IU0) and a library of approximately 450 benzimidazole. The search of potential inhibitors was performed with Glide software (www.schrodinger.com), having the active site as the binding pocket. Compounds were evaluated according to the Drug-like descriptors.

**Results:** The three best molecules were compounds **297**, **298** and **177** which showed binding energies of -8.55, -8.31 and -8.36 kcal/mol, respectively. Compound **297** made hydrogen bond interactions with Asp243, Ser150, Asp194 and salt bridge with Asp137, Asp245 and Asp141. Compound **298** made hydrogen bond interactions with Asp137, Asp243 and Thr257, and salt bridge with Asp141. Finally, compound **177** made hydrogen bonds with Asp194, Val149, Ser150 and Thr148. Predicted drug like properties from these molecules was in the range to be considered as potential drugs.

**Conclusions:** The molecules reported here have the potential to inhibit LmARG, and would be used as starting point for the design of new drugs against leishmaniasis.

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# Association of single nucleotide polymorphisms of the APLN, APLNR and MTHFR genes with the presence of essential hypertension in postmenopausal Yucatecan mestizo women

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ABSTRACT

Hypertension (HT) is defined as a systolic pressure equal or higher than 140 mmHg and a diastolic blood pressure equal or higher than 90 mmHg. In the world, cardiovascular diseases are responsible for about 17 million deaths per year. HT is the main risk factor for morbidity and mortality from cardiovascular disease in postmenopausal women, affecting approximately 60% of those over 65 years old. Estrogen deficiency is associated with impaired of endothelium, because they produce substances that regulate the expansion and contraction of the vessels. Recently, genes as APLN and its APLNR receptor have been linked with cardiovascular diseases such as chronic heart failure and metabolic disorders related to HT. Likewise, there are several SNPs and association studies in the MTHFR gene and the presence of HT. Objective. Analyze whether some SNPs and/or haplotypes in APLN, APLNR and MTHFR genes are associated with the presence of essential HT in postmenopausal Yucatecan mestizo women. Methodology. The polymorphism analysis was performed using the technique PCR TaqMan Allelic Discrimination Assays. A statistical model of multivariate logistic regression was used to identify predictors of HT. Results. In none of the SNPs and under several inheritance model (dominant, recessive and additive) we found association of these with the presence of HT (p-value > 0.05). Likewise, reached statistical power was insufficient (< 0.8). In spite of, in the case of SNP rs1801133 of the MTHFR gene, the p-value is very close to being significant (p = 0.051) in a dominant inheritance model, so we could speculate an association of this SNP as a risk factor for have essential hypertension (OR = 1.76); in contrast to the recessive model, which seems to be protective factor (OR = 0.569). It is noteworthy that this same polymorphism has been associated as a protective factor in Mexican mestizo population. Conclusions. We can conclude that it needs to increase the sample size to certainly find an association of SNPs with the presence of essential hypertension in postmenopausal Yucatecan mestizo women.

# Phenotypic characterization of the maize *med12* mutants

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Mediator is a muiltiprotein complex highly conserved in all eukaryontes, which functions as either a transcriptional activator or repressor. It regulates different developmental and physiological process, such as developmental timing, organogenesis, hormone responses and the response to abiotic and biotic stress.

It is organized into head, middle and tail modules, as well as the additional detachable CDK8 kinase module that consists of MED12, MED13, CDK8 and CYC C. In our lab, we found that maize has two *MED12* genes, *ZmMed12a* and *ZmMed12b*, both of which are expressed throughout the plant. An Ac/Ds reverse genetic strategy was used to generate mutant alleles of *Med12a*; whereas a *Med12b* mutant allele was obtained through the UniformMu Transposon Resource.

We characterized the root system architecture of maize *ZmMed12a* and *b* single mutants. Results show that *ZmMed12* mutants present some traits associated with adaptation to low phosphorus, such as shallower roots and higher acid phosphatase activity, when plants are grown in normal phosphate conditions.

Additionally, we generate a segregating family for both mutant alleles, and measured different traits in the field, and yield after harvest. Our results suggest that maize *med12* mutants affect multiple traits of importance for agriculture.

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# Identification of Lectin from teosinte coleoptile (*Zea diploperennis)*

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We identified a lectin from teosinte coleoptile (*Zea diploperennis*), which is similar to maize β-glucosidase aggregating factor (BGAF). This lectin agglutinate erythrocytes of humans and animals, are also specific for galactose, and it is part of a complex of β-glucosidase. BGAF has been most widely studied.
BGAF belong to a group of lectins identified in the *Poaceae* family known as Monocot chimeric jacalins. These lectins are modular proteins containing a dirigent domain and a jacalin-related lectin domain. A number of studies have shown that these proteins play important roles in plant stress responses and development.
Based on evolutionary relationship between maize and teosinte and similar biochemical properties between BGAF and teosinte lectin, we design primers using sequence information of BGAF to amplify a cDNA of teosinte coleoptile lectin (*Zea diploperennis)* by RT-PCR. We obtain a product with molecular weight of about 1100 bp (molecular weight of BGAF is 1118 bp) in agarose gel and a partial nucleotide sequence. The sequence have similar to sequences encoded by BGAF and related genes. It was 95% identical to BGAF (AF232008), 84% identical to SL (DQ866804.1), 69% identical to Ta-Ja1 (AY372111.1) and 75% identical to JAC1 (DQ243708.2).
Differences between the sequence of BGAF and teosinte lectin is mainly find in the region comprising the dirigent domain in BGAF.

Keywords: teosinte, *Zea diploperennis*, lectin.

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# Evaluation of CDKN3 in cell cycle involvement in cell lines derived from cervical cancer

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**Introduction.**

The CDKN3 gene is upregulated in patients with cervical cancer (CC) and high expression levels are associated with decreased patient survival. In addition, the gene is associated with cell proliferation in CC derived cell lines. CDKN3 is a gene encoding protein phosphatase KAP, and in some cell models has been shown to have a dual function within the cell cycle, as can dephosphorylate cdk2 and prevent the progression of G1 phase to S phase and, moreover, dephosphorylate to CDK1 and promote uutput of M phase. The objective of this study is to determine whether CDKN3 dephosphorylated to CDK2 and CDK1 in CC derived cell lines and at what stage of the cell cycle performs this activity.

**Materials and methods.**

The CC derived cell lines SiHa (HPV16), HeLa (HPV18) and C33 (HPV negative) were cultured in DMEM supplemented. Proteins of the cell lines were extracted with RIPA and were quantified in Nanodrop. Polyacrylamide gels were run at 12%, were loaded with 25 ug of total protein and were transferred to PVDF membranes. For Western blot were used the antibodies against CDKN3, CDK2 phosphorylated, unphosphorylated CDK2 and tubulin as a positive control.

**Results.**

Electrophoretic conditions, transfer and Western blot were standardized. According to the expected size of the protein, polyacrylamide gels were standardized to 12% and electrophoretic shift at 100 V for two hours. Transfer to PVDF membranes was performed at 100 V for 50 minutes in humid chamber. For each antibody used, different dilutions were tested to obtain a satisfactory result, the standardized concentrations were: CDKN3 (1:1000), CDK2 phosphorylated (1:1000), CDK2 unphosphorylated (1:3000) and tubulin (1:10,000). Preliminary results in cell lines SiHa and C33 show the presence of CDKN3 protein (34 kDa) and tubulin protein (50 kDa). Furthermore, the presence of unphosphorylated CDK2 protein (34 kDa) and lack of CDK2 phosphorylated (34 kDa) was observed.

**Discussion.**

CDK2 dephosphorylation could be due to the presence of the protein KAP. To demonstrate this hypothesis, is required a western blot assay after inhibiting the activity of CDKN3 by specific siRNAs. On the other hand, we want to investigate whether CDK2 function is downcast. And if so, we want to investigate whether CDK1 replacing to CDK2, in the G1/S and S-CDK complexes. We still need to investigate whether KAP also dephosphorylated to CDK1 as in other cellular models, and determine if this promotes the exit from mitosis. We are also interested to know the expression of KAP and its targets in cervical tumors.

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# Nuclear RNA extraction as a way to study lncRNAs in *Ustilago maydis*

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*Ustilago maydis,* the causative agent of the corn smut disease, is a dimorphic fungus that experience changes from a saprophytic sporidia to pathogenic hyphae. Its dimorphic transition is governed by a tetrapolar mating-type system that makes mating and pathogenic development concomitant processes. *U. maydis* has been studied since XIX century, with the most impressive achievements reached in the last thirty years. This model organism shares the most desirable attributes as biological system with *Saccharomyces cerevisiae*: yeast-like cells, genome size, chromosome number, growth in synthetic and complex media, and high genetic transformation-frequency among others. *U. maydis* also has attributes that make it unique: this fungus experience dimorphic transition and development from unicellular sporidia to filamentous multicellular mycelia, and harbors in its genome genes that resembles those involved in the development of several diseases and genetic conditions in humans, interestingly, it is devoid of the machinery for miRNA interfering processes. Yet, this fungus is a fertile organism suitable for several more studies, one of these is to find ncRNAs, involved regulatory pathways and those involved in lengthening and maintenance of telomere in lower eukaryotes.

ncRNAs has been described as RNAs with low or lack of translation potential, with length >200nt. Often, finding of this class of transcripts depends in great way on the depth of the RNA-seq, in the way of its capture prior to the RNA fragmentation, and the coverage of the sequencing over regions that encode lncRNAs. Previously it has been reported that natural antisense transcripts (NAT) an intragenic class of ncRNAs do exist in *U. maydis*, and many functional RNAs as TR (Telomeric RNA) subunit, as well as TERRA (**TE**lomeric **R**epeat-containing **R**N**A**) are enriched in the nucleus, and the fact TERRA could be poly adenylated in a minor fraction, as could be other lncRNAs, in this work we attempt to initiate the study of RNA populations harbored in the nucleus of *U. maydis*. We obtained total, nuclear and cytoplasmic RNA from strain 521. The RNAs were purified from DNA, and reverse transcribed using specific oligonucleotide primers for subtelomeric *rqr1* (***r***ec***Q*** ***r***elated) sequences, *trt1* gene, and intergenic RNAs *ter9, ter63* and *ter84*, a set of three non coding genes which were discovered by a combination of informatics and experimental approaches for TR moiety of telomerase. Preliminary qRT-PCR confirmed nuclear enrichment of all genes, except *ter9.* Disruption analysis of *ter84* with a cassette that cleaves the potential template CCCUAA from the non-coding gene gave results non-compatible with disruption of TR subunit of telomerase. Colonial and cellular morphology was indistinguishable from that of WT 521, as well was its growth kinetics. Possible involvement in pathogenicity was inconsistent to date. Our approach to extract nuclear RNAs was successful as the results could be the start point to analyze other species of nuclear RNAs.

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# Tumor suppressor miR-29c regulates radioresistance in lung cancer cells

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Radiotherapy is an important treatment option for non-small cell lung cancer (NSCLC) patients. Despite the appropriate use of radiotherapy, radioresistance is a biological behavior of cancer cells that limits the efficacy of this treatment. Deregulation of microRNAs (miRNAs) contributes to the molecular mechanism underlying resistance to radiotherapy in cancer cells. Although the functional roles of miRNAs have been well described in lung cancer, their functional roles in radioresistance are largely unclear. In this study, we established a NSCLC radioresistant cell line Calu-1 (Calu-1RR) by continuous exposure to therapeutic doses of ionizing radiation as a model to investigate radioresistance-associated miRNAs. Our data show that 50 miRNAs were differentially expressed in Calu-1RR cells (16 up-regulated and 34 down-regulated); furthermore, well-known and novel miRNAs associated with resistance to radiotherapy were identified. Gene ontology and enrichment analysis indicated that modulated miRNAs might regulate signal transduction, cell survival and apoptosis. Accordingly, Calu-1RR cells were refractory to radiation by increasing cell survival and reducing the apoptotic response. Among deregulated miRNAs, miR-29c was significantly suppressed. Reestablishment of miR-29c expression in Calu-1RR cells overcomes the radioresistance through the activation of apoptosis and down-regulation of *BCL2* and *MCL1* target genes. Analysis of The Cancer Genome Atlas (TCGA) revealed that miR-29c is also suppressed in tumor samples of NSCLC patients. Notably, we found that low miR-29c levels correlated with shorter recurrence-free survival of NSCLC patients treated with radiotherapy. Together, these results indicate a new role of miR-29c in radioresistance, highlighting their potential as a novel biomarker for outcomes of radiotherapy in lung cancer.

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# Functional subfunctionalization of branched chain aminotransferases through diversification of transcriptional regulators and chromatin organization in the yeast *Saccharomyces cerevisiae*

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It has been previously shown that the ancestral type *Kl*Bat1 orthologous BCAT is a bifunctional enzyme, which participates in the biosynthesis and catabolism of branched chain aminoacids (BCAAs). This dual role has been distributed in *S. cerevisiae* Bat1 and Bat2 paralogous proteins, through diversification of the expression profile: *BAT1* is highly expressed under biosynthetic conditions, while *BAT2* expression is highest under catabolic conditions. Here we have identified the transcriptional modulators whose action determines opposed BAT1 and BAT2 expression. Our results show that, Put3 and Leu3 play dual roles. Put3 determines BAT1 VIL-dependent repression and BAT2 VIL-dependent activation, while Leu3 determines glutamine-dependent BAT2 repression, and BAT1 glutamine-depenent activation. Chromatin remodeling accompanies differential expression on biosynthetic or catabolic conditions. The ancestral LkBat1 or KlBat1 orthologous BCATS are bifunctional enzymes, which participate in the biosynthesis and catabolism of branched chain aminoacids (BCAAs). Their expression is constitutive and is not modified under biosynthetic or catabolic conditions, accordingly chromatin organization is similar under both growth conditions. These results indicate that acquisition of Put3 and Leu3 trans-acting elements and their corresponding *cis*-acting elements, has been determinant for retention, expression diversification and distribution of the ancestral bifunctional capacities in two paralogous genes .

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# Genomic and functional studies of miRNA regulation of Arabidopsis embryogenesis

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Embryo development in flowering plants is known to be regulated by transcription factors and proteins involved in the metabolism and transport of hormones such as auxin. miRNAs, master regulators of gene and protein expression, are known to play an important role in pattern formation and morphogenesis during vegetative and reproductive development. Since the pattern of vegetative and adult tissues is originally established in the embryo, we are interested in identifying and studying the role of miRNAs and its targets involved in patterning of Arabidopsis embryos. miR156 targeted SQUAMOSA PROMOTER BINDING PROTEIN (SBP/SPL) transcription factors regulate two developmental transitions in shoot—the juvenile-to-adult vegetative transition and the vegetative-to-reproductive transition. Here, we have studied the role of miR156 and SPL transcription factors in embryo development. Our data reveal that *SPL2, SPL3, SPL9, SPL10, SPL11,* and *SPL13* are repressed by miR156a/c during embryo development. We found that some miR156- targeted SPL genes regulate early formative divisions required for embryo patterning, and that others can interfere with pattern formation when miR156 regulation is removed. Our data suggest that the role of miR156 in early embryo development is to repress some *SPL* genes, so that pattern formation can proceed normally.

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# ASSOCIATION OF *VDR, KLOTHO*, *CYP27B1*, *PTPN22* and *AGTR2* SNPs WITH CLINICAL CHARACTERISTICS IN TURNER SYNDROME PATIENTS.

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*Turner syndrome* (TS) is a genetic *disorder* that affects about *1* in 2500 newborn *girls,* TS results when one normal X chromosome is present in a female's cells and the other sex chromosome is missing or structurally altered. TS patients are characterized by short stature, lack of normal pubertal development due to gonadal dysgenesis, and other clinical signs that impact the quality of life including cardiac and kidney malformations, autoimmune thyroiditis and predisposition to obesity and glucose intolerance. The presence of these defects varies from patient to patient. Associations of single nucleotide polymorphisms (SNP's) and clinical manifestations in TS patients are reported in literature. **OBJECTIVE**: Study the association between SNP´s and clinical features in patients with Turner Syndrome. **METODOLOGY**: We included 67 patients with TS and 80 control females. Karyotype study of all participants were performed. DNA was extracted from peripheral blood with salting out technique. Genotyping of *VDR* (rs7975232), *KLOTHO* (rs9536282), *CYP27B1* (rs4646536), *PTPN22* (rs1599971) y *AGTR2* (rs5194) was realized by the KASP assay (LGC Genomics, Beverly, Ma. USA http://www.lgcgenomics.com/). Subsequently an association study of clinical manifestations as thyroiditis, heart and kidney malformations in patients with TS and the SNP's found was performed. Hardy-Weinberg equilibrium was obtained by Fisher's exact test. Single variant association with the clinical manifestations was analyzed by an Armitage's trend test and gene-gene interaction analysis was performed by the multifactorial dimensionality reduction method. **RESULTS**: The distribution of karyotypes in our study population was 65,X in 18%, 17% mosaics and 25% structural alterations. In the single variant analysis, a significant association was found in between variant rs956282 (*KLOTHO*) and renal malformation OR 18.6 (C.I. 95% 0.91-500.38) p=0.015. Gene-gene interaction was observed between variants rs9536282-*KLOTHO*, rs5194-*AT2R*, s1599971-*PTPN22* to a greater risk to TS, Testing accuracy (TA) = 0.699, Cross validation (CV) = 10/10, OR 8.0 (C.I. 95% 3.5-18.04), p=0.0007. Gene-gene Interaction was also observed for variants in genes involved the metabolic pathway of vitamin D as rs9536282-*KLOTHO*, rs4646536-*CYP27B1*, rs7975232-*VDR*, and a greater risk to TS, TA= 0.6526, CV= 10/10, OR 3.72 (C.I 95% 1.16-11.95) p=0.0001.
**CONCLUSION**: The frequency of Karyotypes in TS patients are very similar to those reported in other studies. In patients with TS presenting the T allele (homozygote state) of rs9536282 (*KLOTHO*), have greater susceptibility to renal malformation. Gene-gene interaction between the three variants located in genes involved in the vitamin D pathway (rs9536282-KLOTHO), (rs4646536-CYP27B1), (rs7975232-VDR) are associated to a greater risk to TS. It is indicating that the metabolic activity of vitamin D may be relevant for clinic features in TS patients. Financial support: FONCICYT 95419 CONACYT 142040. Fondos Federales INP 84/2010.

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# miR34a as possible regulator of ubiquitin ligase E6AP in a model of HPV18 E6 overexpression.

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Human papillomaviruses are the main ethiologic factor for Cervical Cancer (CC). The HPV E6 gene encodes an oncoprotein that is expressed at early stages of viral infection and recognizes numerous host proteins leading to cell transformation. The E6 oncoprotein binds a cellular ubiquitin ligase, E6AP, resulting in the recruitment, polyubiquitination and degradation of the tumor suppressor p53, as well as several other cellular proteins. This E6/E6AP complex is required for E6-induced degradation of p53. Negative regulation of E6AP could prevent formation of this complex and therefore lead to p53 upregulation. microRNAs (miRNAs) have emerged as important elements of gene regulation through post-transcriptional level because they can inhibit gene expression through transcriptional repression and degradation of mRNAs. Using bioinformatic tools we found that E6AP is a predicted target for mir-34a; therefore its ovexpression could possibly be regulated by this mRNA. With the aim of validating this interaction, HeLa cells (cervical carcinoma-derived, HPV18+) were transfected with miR-34a mimic. We found differential E6AP expression in transfected HeLa cells compared to non-transfected cells. This evidence hints at therapeutic strategies based on impeding p53 degradation in CC.

Keywords: Papillomavirus, oncoprotein, E6, miRNAs, cervical cancer, E6AP.

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# Risk Factors Associated with Lack of Weight Reduction in Morbidly Obese Patients

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**Introduction**: In Mexico, morbid obesity has increased to reach 3% of the population. Morbidly obese patients should reduce 10% of their weight excess previous bypass surgery. Initial treatment consists of diet and exercise plan. However not all patients reduce excess weight required. The aim of this study was to determine incidence of failure and risk factors associated with lack of weight loss in morbidly obese patients.
**Methods**: Prospective cohort of morbidly obese patients treated at Morbid Obesity Multidisciplinary Clinic from Hospital Specialties, CMN Siglo XXI. Cohort integration was performed in pre-consultation, all patients with BMI ≥ 40 kg/m$^2$ were included. All patients received a diet and exercise plan designed *ad hoc* to reduce 10% of excess weight by multidisciplinary team. Final measurement was carried out at six months of the pre-consultation. Patients with 10% reduction of overweight were considered with weight loss. Categorical variables were associated calculating risk ratio (RR) with confidence intervals at 95% (CI95%). Quantitative variables, were analyzed with U-Mann-Whitney. To determine independent risk factors, statistically significant variables and age were adjusted in a multiple logistic regression model.
**Results**: One hundred ninety-four patients were included in the study. Most were women (78%). Only 61 (31.4%) patients reduced 10% of excess weight. Hypertension was associated as a risk factor for not weight reduction (RR = 1.911, CI95% CI [1.239-2.950]), while higher BMI (p = 0.048), higher FSH concentration (p = 0.028), higher insulin concentration (p = 0.036), lower albumin concentration (p = 0.004) and total bilirubin (p = 0.029), were also associated. In multivariate analysis, higher plasmatic concentrations of insulin (OR = 1.042, CI95% [1.001-1.085]), FSH (OR = 1.092, CI95% [1.013-1.176]) and albumin (OR = 0.063, CI95% [0.008-0.507]) are associated independently with weight reduction.
**Conclusion**: Insulin, FSH and albumin plasmatic concentrations are independently associated with reduced body weight in morbidly obese patients.

# "Gene expression profile of HSP90AA1 y HSP90AB1 in clear renal cell carcinona (CRCC) and clinical implications".

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**Introduction:** The Heat Shock Proteins of 90kDa (Hsp90) are encoded by a family of genes needed in cell survival, being lethal his knockout. In the genes subfamily of Hsp90, the citosolic isoforms are HSP90AA1, wich encodes the inducible isoform Hsp90α and HSP90AB1 which encodes the constitutive isoform Hsp90β. In cancer, the activity of many oncogenes and tumors supressors are regulated by the interactions with Hsp90. In many types of cancer has been identified that Hsp90 is overexpressed two to ten times, associating with a poor pronostic for the patient. Recent studies shown that exist a transcriptional regulation between HSP90AA1 and HSP90Ab1 genes. In lung cancer the overexpression of HSP90AB1 gene has been associated with lymphatic invasion and lower survival. Interestingly, Hsp90 has not been identified in renal carcinoma (RC) and even less each isoform roll.
**Objective**: Determinate the clinic implication of gene expression profile of HSP90AA1 and HSP90AB1 in renal tissue samples from patients with CRCC.

**Materials and methods:** The study includes a 20 patients cohort diagnosticated with Clear Renal Cell Carcinoma (CRCC), of which the total RNA from renal tumoral tissue and adjacent tumor tissue was extracted from Trizol method. The analysis of the expression of the genes HSP90AA1and HSP90AB1 was determinated by RT-PCR. The semi-quantitative analysis was performed using the ImageJ software. The clinical variables that were associated with each one of the expression profiles of HSP90AA1 and HSP90AB1 were: metastases at diagnostic, disease progression and the overall survival.

**Results:** The expression profile obtained by the relation HSP90AB1/HSP90AA1>0 identified a subgroup of patients with CRCC with metastatic stage and lower survival, evaluated 24 months after nephrectomy. For first time it´s reported the subexpression of inducible isoform HSP90AA1 in patients with CRCC asociating with CR progression.

**Conclusions:** A first analysis of our patients group with CRCC show that the overexpression of HSP90AB1 isoform is associated with lower survival. This suggets that in the future,an increased profile in the expression of HSP90AB1 isoform could help to diagnosis and staging in patients with CRCC . With the variables studied, the gene expression of HSP90AA1 didn't show any association. Our investigation group is working in the genes expression profile of HSP90AA1 and HSP90AB1 increasing the number of patients with CRCC.

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# Expression Profile of microRNAs in Small Cell Lung Cancer tumors.

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Small Cell Lung Cancer (SCLC), an aggressive neuroendocrine tumor characterized by high proliferation rates and early metastasis, has an overall poor prognosis even after conventional treatment with chemotherapy and radiotherapy. Although decades of work have led to a better understanding of the genetic abnormalities behind SCLC, the mechanisms promoting its aggressive phenotype have not been fully elucidated. Most SCLC cases are inoperable and biopsies to study its biology are rarely obtainable. Moreover, there is an urgent need to identify early diagnostic markers and more effective therapeutic strategies to treat SCLC. MicroRNAs (miRNAs) are known to be dysregulated in tumorigenesis and a few studies have implicated them in SCLC pathogenesis. However, the role of miRNAs as predictive markers of SCLC tumors has not been thoroughly evaluated.

In this study, we identified miRNAs differentially expressed in SCLC tumors from 5 patients when compared to their matched, normal tissue, using TaqMan Low Density Arrays (TLDA) and real-time PCR (qRT-PCR). Additionally, we validated their expression level in the SCLC cell lines NCI-H69 and NCI-H128 using qRT-PCR.

We found a signature of 18 miRNAs differentially expressed in SCLC tumors, out of which we chose a panel of 5 miRNAs (miR-301a, miR-301b, miR-25a, miR-210, and miR-143) whose level of expression in biopsies correlated with their level of expression in NCI-H69 and NCI-H128, with the exception of miR-25a. The miRNAs miR-301a, miR-301b and miR-210 were found to be up-regulated in tumors and cell lines, whereas miR-143 was found to be down-regulated. Collectively, our findings provide an extensive analysis of the miRNA expression pattern in SCLC which could be used as potential therapeutic and prognostic predictors of this disease.

# Evaluation of gamma and delta tubulins in cervical cancer progression.

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**Resumen (ingles):**

Cervical cancer is the second cause of death in Mexican women population. Infection with high-risk human papillomavirus (i.e. HPV-16 and 18) has been causally associated with the onset of cervical cancer. Microtubule system is a candidate highly used to treat cancer by using drugs such as etoposide or docetaxel seeking to avoid replication and mobility of cancer cells by the catastrophe of the microtubules. However with the passage of time they are still discovering mechanisms by which the microtubule system is involved in the development of cancer being affected various signaling pathways. These pathways are regulated via microtubule and its accessory proteins like cell division that is dependent on mitotic spindle, in apoptosis structures surrounding the nucleus and cytoplasm are formed to prepare cell death. On the other hand in the processes of migration and invasion an arrangement of cytoskeleton leads to cell movement. Which leads us to propose that the expression of tubulin isoforms probably allows processes involved in carcinogenesis. In this work we shown that gama tubulin expression increase through cervical intraepithelial neoplasia I (NICI), II, III and cervical cancer. So far the expression of delta tubulin in the progression of cervical cancer is not clear. Hence the expression of gama tubulin could be used as a marker for cervical cancer diagnosis..

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# A *Phaseolus vulgaris* annexin modulates the rhizobial infection and the nodulation process

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Legumes establish a symbiosis with $N_2$-fixing soil bacteria known as rhizobia. This process initiates with a molecular dialog among the symbionts. *Rhizobium* secretes to the rhizosphere lipochitooligosacharide signals, known as Nod factors, which induce root hair deformation, an increase in the levels of calcium, reactive oxygen species production, ion influxes/effluxes, cell-membrane depolarization, cytoplasm alkalinization, perinuclear calcium oscillations, cortical cell divisions and transcriptional activation of plant genes known as nodulin genes (1).

Plant annexins are a family of calcium- and membrane- binding proteins involved in growth and development, as well as in several abiotic and biotic responses. Evidence supports that annexin can modulate $Ca^{2+}$ signaling by creating ion channels, and regulate the production of $H_2O_2$ (2, 3). Herein, thirteen annexin genes were found in *P. vulgaris* genome. Out of these, *PvAnn93* transcript is highly accumulated in root hairs relative to root apex and stripped roots. To gain insight into the role of this annexin during the nodulation process in *P. vulgaris*, *PvAnn93* transcript accumulation levels were monitored in the roots after *R. tropici* infection at early (3, 5, 7 and 9 days post inoculation, dpi) and at late stages (10, 14, 17 and 21 dpi). Loss-of-function of *PvAnn93* in transgenic roots inoculated with rhizobia diminished the efficiency of ITs progression and the expression of *NIN* and *Enod2*, genes involved in early nodulin signaling. Moreover, a reduced number of nodules and less nitrogen fixation levels were found at 21 dpi. Together these data suggest that loss-of-function of *PvAnn93* limits ITs progression, resulting in a delayed nodule development and functioning.

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# Regulator ThnR and ABC transporter are necessary to immunity of *Bacillus thuringiensis* against Thurincin H(m)

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*Bacillus thuringiensis* is a gram-positive bacterium with insecticidal properties owing to its synthesis of Cry and Cyt protein protoxins. *B. thuringiensis* also produces small peptides called bacteriocins with activities against clinically significance pathogens. At present, the N-termini of eleven bacteriocins of *B. thuringiensis* have been partially sequenced, but only the primary structure of thurincin H and thuricin CD are known. These bacteriocins are ribosomally synthesized, post-translationally modified and activated for secretion, they are classified as sactipeptides (cause of theirs bond sulfur-$\alpha$ carbon), a newly recognized emerging class of molecules with diverse bioactivities.

The specific mechanisms responsible for the regulation of *B. thuringiensis* bacteriocins expression and immunity have been little studied. Recently it was demonstrated that determinants responsible for synthesis, regulation, post-translational modification, processing, and self-immunity to thurincin H and thuricin CD are organized in a cassette of tightly linked gene clusters, similar to the observed in other bacteriocins. In 2007, we reported that a Mexican strain of *B. thuringiensis* subsp. *morrisoni* (strain LBIT 269) synthesizes a bacteriocin that we initially called “morricin 269” and hereafter “thurincin H(m)”. This bacteriocin shows the same genetic cluster to Thurincin H. The purpose of this work was to understand the immunity mechanism used by the bacterium to resist its own bacteriocin. The gene cluster possesses three ORF that could be related with immunity. *thnD*, *thnE* and *thnI*, the first two are the ATP binding domain and permease protein of ABC transporters and the last is probably a immunity protein. These genes were cloned and transformed into a sensible *B. thuringiensis* (LBIT 404). When this strain was challenged with Thurincin H(m) the strain that harbor *thnD-thnE* genes were a little resistant and with *thnR-thnD-thnE* were completely resistant. The three genes are necessary to provide immunity. Construction with *thnI* does not have any effect.

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# Analysis of expression of TFIIB1 transcription factor in *Arabidopsis thaliana* and *Phaseolus vulgaris*

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Plants are organisms that are exposed to variations of environmental factors that can affect their growth and productivity; these adverse conditions are known as stress, which can be classified into biotic and abiotic. Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agriculture and result in the deterioration of the environment. Thus, plants have developed response mechanisms that allow them to survive among stress conditions such as the synthesis of LEAs proteins (Late embryogenesis abundant), chaperones (Heat shock proteins) , sugars (trehalose) , metabolites and compatible solutes ( glycine betaine and proline). The synthesis of molecules to contend with stress conditions, involves the expression of genes that are regulated by transcription factors (TFs). The general transcription factor TFIIB is a highly conserved monomer essential for the assembly of preinitiation complex furthermore it have been shown that expression of the transcription factor TFIIB is involved with the stress response in *Saccharomyces cerevisiae*, *Citrus clementina* and *Oreochromis mossambicus*. In particular, *A. thaliana* has at least 14 members belonging to the gene family TFIIB, including TFIIB1, it is known that this TF is involved in pollen tube growth, seed formation and the stress response. The RT- PCR analysis showed that TFIIB1 presents alternative splicing due to third intron retention in drought stress conditions and salinity. However, it is unknown whether the constitutive overexpression of TFIIB1 canonical and alternative participates in the regulation of stress response genes to contend with abiotic stress. Based on the background of stress response of some members of the family TFIIB, it is important to study this family of TFs in plants of agricultural interest, as in the case of *Phaseolus vulgaris* (bean) whose genome has been sequenced. Overexpressing TFIIB1 *A. thaliana* homozygous plants were obtained with the pBin35::TFIIB1 construct and transcriptome analysis was performed using microarrays.On the other hand; in *P. vulgaris* at least five members of the family TFIIB were identified in the bean genome using the databases PvGEA and Phytozome. Names for each of the TFs were assigned based on a multiple alignment with the sequences of *A. thaliana* TFIIB family. Quantitative RT-PCR assays were performed to analyze the expression patterns of these TFs in different tissues of bean plants grown under normal conditions and salinity, drought, cold and oxidative stress. Results showed that under salt and oxidative stress some TFs were overexpressed, unlike, under drought they were repressed. Besides, differential patterns of expression were observed in specific tissues.

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# Preliminary Study about the effect of Maternal Obesity on Global DNA Methylation in Placenta.

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The current epidemic of obesity represents a major global health problem worldwide. In Mexico the obesity prevalence in pregnant women is about 60%. Overweight and obesity during pregnancy are associated to development of obesity and metabolic diseases in later-life of the offspring[1]. The prenatal exposure to obesity impacts directly the fetal programming and the development plasticity, representing an adverse risk factor involved in several diseases. Animal models indicate that many activated pro-inflammatory pathways by the fetal exposure to excess blood lipids, impact the substrate metabolism and mitochondrial function, affecting the organ development and the response to the postnatal environment[1]. Related to this, the emerging field of epigenetics is recognized to have an important but still poorly defined role in fetal metabolic programming.

Epigenetic mechanisms are heritable marks that regulate gene-environment interactions. DNA methylation of the CpG islands is considered the major epigenetic event that can influence the regulation of gene expression[2]. Little is known about the precise mechanism by which differences in nutrient exposure can alter epigenetic marks in humans, although recently it has been demonstrated that prenatal conditions, such as nutrient restriction, can epigenetically modify gene expression by altering the methylation level of DNA in gene promoter regions. However, the maternal obesity combined with normal changes in maternal metabolism, may increase inflammation and serum lipids, which can have profound effects on the fetal programing.

**Objective:** The aim of this work was to identify and compare genome-wide DNA methylation variation in placental tissue from obese and non-obese individuals.

**Methods:** A convenience sample of term pregnancy women was recruited for this prospective study since the first trimester. They were separated in two groups: obese (n=5/group) and non-obese (n=4/group), according to their pre-gestational body mas index (pBMI). At the end of pregnancy placentas were collected and the DNA was purified using a modified protocol of DNAzol™ (Invitrogen). Genome-wide DNA methylation was evaluated using the Illumina Infinium DNA methylation 450K bead-based arrays. Genome-wide DNA methylation analysis was done using the Genome Studio DNA methylation module (Illumina). **Results:** The differential analysis of 485,000 CpG islands along the human genome between placentas from obese and non-obese women showed that some of them change significantly their methylation profile in specific regions within the genome as a response to obesity. Our preliminary analysis also showed an increase in the number of GpG islands hyper-methylated within all the genome.

**Conclusion:** This preliminary and prospective study showed that maternal obesity impacts the DNA methylation profile of several genes in placental tissue increasing the global DNA methylation, offering the possibility to select genes that change significantly their methylation profile to explore their implication in different metabolic pathways or in several diseases.

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# Bariatric surgery-associated DNA methylation remodeling, in adipose tissue from obese patients.

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***Introduction.*** 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 [3]. Recently it has been reported that epigenetic alterations, especially DNA methylation, may have an important role in the pathogenesis of metabolic diseases. Bariatric surgery is a radical intervention in the treatment of obesity and co-morbidities and there is still little information about epigenetic remodeling and its relation to the improved metabolic health that is associated with weight reduction after surgery.

***Material and Methods.*** 36 patients with obesity submitted to bariatric surgery were included. All patients were Mexicans, with similar baseline characteristics between groups, 17 with diabetes (OD) and 19 without (OB). A subcutaneous adipose tissue (SAT) biopsy was collected at the surgery time. Global DNA methylation profiles were analyzed using Illumina Infinium HumanMethylation450K Bead Chip. Methylation profiles were contrasted between groups the day of intervention and six moths after. DAVID was used to enrichment analysis. We searched for differentially methylated CpG sites (DMCs) in OD, comparing with OB patients in the day of surgery. A CpG site was defined as DMC when a p value<0.05 and at least 0.05 absolute mean methylation difference. Six months after the surgery, a SAT biopsy was collected for all patients. DNA methytilation profiles were evaluated and compared with those at the moment of surgery.

***Results.*** We found diverse DMCs in OD compared with OB patients at the time of surgery. Analysis of DNA methylation after 6 months of surgery, showed changes in profiles in both OD and OB patients. Many of DMCs reversed their differences between both groups. We also found sites with a high correlation between DNA methylation level and clinical parameters

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# Transcriptomic analysis of the adaptation of *Ustilago maydis* to pH changes

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All organisms are exposed in the environment to different chemical, physical and physicochemical factors that may affect their growth and development, among them different compounds, nutritious or inhibitors, temperature, pressure, and pH. Accordingly, their survival will depend directly on their capacity of adaptation to changes in these factors. Thus, it is known that fungi possesses one known mechanism involved in their adaptation to an alkaline pH change, the Pal/Rim pathway.
In order to determine if the phytopathogenic Basidiomycota *Ustilago maydis* posseses this single mechanism, and the genes involved in the process, we analyzed its transcriptome during its adaptation to changes of pH when transferred from a neutral to an acidic, or to an alkaline pH. Our analysis involved the wild type strain that grows yeast-like at neutral or alkaline pH, and as mycelium at acid pH; and, as controls, mutants that grow as yeast or mycelium at either pH. This way, we identified a total of 301 specifically regulated by the change to pH 3, 162 up-regulated and 139 down-regulated. Functional categorization (FunCat) of the genes showed that metabolism, transport, cellular defense and environment interaction categories were the ones with the greater number of genes. On the other side, 797 genes were specifically regulated by the change to pH 9. Of these, 335 genes were up-regulated, and 462 genes were down-regulated. In this case, the categories with the higher number of genes were metabolism, transcription, transport, proteins with binding function or cofactor requirement, and cell rescue. Interestingly, many of these genes were not regulated by the Pal/Rim pathway. These results indicate that *U. maydis* (and possibly other fungi) undergoes a more drastic modification on its transcriptional program in order to adapt to alkaline conditions, compared to its adaptation to acid pH. In addition they demonstrate that adaptation to an acid pH does not involve the Pall/Rim pathway, and that adaptation to alkaline pH probably involves other mechanism(s), besides the Pal/Rim pathway.

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# The role of dAtrx ATPase in the telomeric maintenance of *Drosophila melanogaster*

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*Drosophila* telomeres are composed of three regions, the TAS (telomere associated sequences) region, the HTT array that contains three retrotransposons Het-A, TART and TAHRE and finally a region at the end of the telomere called the CAP. This organism maintains its telomeres through a couple of telomerase-independent pathways: homologous recombination and retrotransposition of the HTT array. The retrotransposition involves the transcription of the retrotransposon sequences (some of which encode a GAG protein and a reverse transcriptase) and insertion of DNA copies of retrotransposons in the genomic DNA, this pathway is regulated by siRNAs in somatic cells[1] and by piRNAs in the germinal line[2]. The protein Atrx is a chromatin remodeler composed of an ADD and a helicase-ATPase (SNF2) domains. The ADD domain recognizes the H3K9me3 and unmodified H3K4 and directs the protein to heterochromatic and repetitive regions [3]. The SNF2 domain of Atrx is necessary for the correct deposition of the histone variant H3.3 in telomeres and pericentromeric regions mediated by the histone chaperone DAXX. In mammals Atrx is required for the efficient silencing of IAP retrotransposons and the negative regulation of the Alternative Lengthening of Telomeres pathway (ALT) in cells[4].

In *Drosophila* the dAtrx domains (ADD and SNF2) are encoded in two separate genes *datrx* and *dadd1*. Previously in our lab we have described that dAtrx and dAdd1 interact physically and genetically and work together in heterochromatin maintenance. It is known that both proteins are localized in the telomeric region named TAS and are capable to interact with HP1a. Considering its capacity to regulate retrotransposon sequences, its localization in telomeric regions and its interaction with heterochromatin proteins, we hypothesized that dAtrx or dAdd1 could be involved in the telomeric maintenance pathways of *Drosophila*.

Using Real Time PCR we analyzed the length of telomeres through the quantification of DNA copy number and transcript abundance of telomeric retrotransposons in somatic cells and germ line of adult males. Using mutant alleles of *datrx* and *dadd1* that affect the wildtype function of the proteins we observed that the absence of both proteins increases the lengthening of telomeres and transcript abundance of the HTT array in somatic cells. These leads to chromosomal aberrations and genomic instability. Whereas in the germ line it only affects the transcript levels of one retrotransposon and the copy numbers remain unaffected which indicates the participation of other pathways (probably mediated by piRNA) to ensure the correct telomere maintenance of these type of cells.

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# Ribosomal protein S1 is required for recognition of downstream adenine- or uracil-rich mRNAs by 30S subunit

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Adenines downstream of the start codon promote protein synthesis and the mRNA binding to 30S ribosomal subunit. 30S subunit protein S1 is known for its high affinity for single-stranded AU-rich RNA stretches. Additionally, S1 is involved in the docking of structured mRNAs to the 30S subunit. Thus, protein S1 could be pivotal for recognition of the downstream A- or U- rich mRNAs by 30S subunit.

In this work, we analyzed the role of ribosomal protein S1 in the binding of low structured mRNAs (containing adenine, guanine or uracil tandems downstream of the start codon) to 30S ribosomal subunit in the formation of ternary complexes with initiator tRNA. Synthetic transcripts with similar thermodynamic stability and containing adenine, guanine or uracil tandems a positions 2-3 and wild type and S1-deficient 30S subunits were prepared. The messengers were 5' labeled with $^{32}P$ to quantify the messenger bound to the ternary complex. As expected, synthetic A- and U-rich messengers bound stronger to 30S subunit than messengers with guanines at the same positions. Interestingly, the enhanced binding of the adenine- or uracil-rich mRNAs was reduced to a greater extent when the ternary complex was S1-deficient. The binding affinity was restored to wild type levels when S1-deficient subunits were reconstituted with purified ribosomal protein S1 but not by an S1 mutant lacking domains 1-3 (S1Δ1-3).

The formation of binary complexes between wild type or mutant S1 proteins and the synthetic mRNAs was analyzed using gel shift assays. R-protein S1 alone showed a higher affinity for A- or U-rich mRNAs compared with G-rich RNA messengers like in the primary complex assays. Interestingly, deletion of the first 2 or 3 S1 domains abolished its binding to U-rich mRNA, and the loss of the first three domains also prevented its binding to U-rich mRNA. S1 did not show binding to G-rich mRNA.

These results indicate that the enhanced binding of A- or U-rich mRNAs to 30 ribosomal subunits is due to the direct interaction of these messengers with ribosomal protein S1.

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# Analysis of the regulation exerted by mir-26a on the *rb1* and *apc* messengers associated to colorectal cancer.

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Colorectal cancer refers to any malignancy in the tissues of colon, rectum and appendix. It affects one in three men and one in four women, and occupies the third and fourth spots in incidence and mortality respectively. Most colorectal tumors bear mutations in tumor suppressor genes such as adenomatous polyposis coli (APC) and retinoblastoma (Rb1). APC regulates functions such as cell adhesion and plays an important role in Wnt**/ß-**catenin signaling pathway while Rb1 is key to the G1-S transition. Deregulation of these genes leads to the formation and progression of neoplasia. An important part in this deregulation is owed to microRNAs, which are 20-nucleotide-long, small, noncoding RNA molecules with nuclear processing and cytoplasmic maturation that exert negative post-transcriptional regulation on their target messengers. In this work we examined whether there a regulation of miR26a upon the APC and Rb1 in an *in vitro* model. The full-lenght 3'UTR regions of Rb1 and APC messengers were amplified and subsequently ligated to the pMIR-REPORT expression vector for luciferase reporter assays. Luciferase activity decreased significantly in the case of the RB1 gene. No regulation was observed in the case of APC gene. Through bioinformatic analysis of the 3'UTR regions, we found that the miR-26a interaction region of the Rb1 3'UTR has more nucleotide available for hybridization compared to the same region of APC 3'UTR. This work sheds new information on the factors that influence the tight regulation exerted on the APC and Rb genes during CRC development broadening our perspective of such a complex disease.

**Key words**: colorrectal cáncer, microRNAs, 3´UTR, Rb1 and APC gene.

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# The strands 5p and 3p of the members of the family miR-34 have differential effects on SiHa cell proliferation and migration

Sergio Córdova-Rivas, José Luis Ruiz Carrillo, Ruth Cristina Rodríguez Garces, Hiram Hernández López and Jesús Adrián López

MicroRNAs (miRNAs) play pivotal roles in controlling cell proliferation, apoptosis and invasion. Aberrant miRNA expression is now recognized as a molecular mechanism for many human tumors including cervical cancer. Infection with high-risk human papillomavirus (i.e. HPV-16 and 18) has been causally associated with the onset of cervical cancer. Furthermore, expression of cellular miRNAs has been linked to cervical cancer independently or associated to HPV expression. HPV-16 E6 protein inactivates and destroys p53 leading to a p53-null phenotype. P53 induces the transcription of the members of miR-34 family formed by miR-34a-5p, miR-34a-3p, miR-34b-5p, miR-34b-3p, miR-34c-5p and miR-34c-3p. This family is transcriptionally regulated by p53 in response to cell damage and oncogenic stress. The ectopic expression of the miR34 family members recapitulates the biological effects of p53. In this work we analyze the function of miR-34 members on SiHa cell proliferation. Over-expression of miR-34a-5p and miR-34a-3p in cervical carcinoma cells causes 30% inhibition of SiHa cell proliferation. MiR-34b-5p mimic causes 30% inhibition of SiHa cell proliferation, however, miR-34b-3p mimic recorded no effect. MiR-34c-5p and miR-34c-3p reach 85% inhibition of SiHa cell proliferation. While in migration only miR-34a-5p and miR-34c-3p present effect. Our results show that the miR-34 family regulates cell proliferation and migration at different extent and this knowledge could be used as therapeutic or diagnostic/prognostic tool in cervical cancer.

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# mir- 26a, epigenetic regulator associated with colorectal cancer

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Colorectal cancer (CRC) is the fourth most common cause of death cancer-related worldwide. It has been observed that patients with chronic inflammatory bowel disease are more likely to develop this cancer. In addition, studies have shown decreased in the expression of tumor suppressor genes p53, GSK3B , APC , CDKN1A , PTEN and Rb1 . Moreover, new evidence suggests that microRNAs are oncogenic and promote tumor growth and development. 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 developed an inflammation-associated mouse CRC model by the method of Azoxymethane / Dextran Sodium Sulfate; neoplasia development was validated through histological sections. We found overexpression of mir-26a corresponding with decreased GSK3B, APC, PTEN and Rb1 mRNA and protein by qRT-PCR and Western blot respectively; we obtained consistent results from SW620 cell line. To determine whether miR-26a regulated to these genes, we were set up luciferase reporter assays cloning the specific mir-26a interaction region from each 3'UTR into the pMIR-Report plasmid. Luciferase expression was decreased in all constructs, more significatively in PTEN and GSK3B. Finally, we found that overexpression of mir-26a increased proliferation and migration in CCR cells, as suggested by the direct regulation exerted by mir-26a on the GSK3B, PTEN and Rb1 at messengers. In conclusion, mir-26a promotes CCR development by inhibition of GSK3B, PTEN and RB1. These signaling molecules constitute tantalizing targets for prevention or treatment of CCR.

Area: Biomédicas

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# The role of Tmk3-Atf1 pathway on blue light responses in *Trichoderma atroviride*

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The Fungi possess a conserved MAPK pathway (Hog1/Sty1/SakA) related to stress responses. This pathway regulates gene expression through some transcription factors, including a basic-leucine zipper (bzip) protein called Atf1 (homologous to Atf2 in humans). In *T. atroviride*, the MAPK Tmk3 (Hog1/Sty1/SakA) regulates some light responses, like reproduction and regulation of genes dependent of light (*blu*1, *grg*2, *hsp*1). In addition, we demonstrated that Atf1 regulates photoconidiation in *T. atroviride*. To better understand this pathway and its relationship with blue light, in this work we analyzed the transcriptome regulated by light in mutants lacking *atf*1 and *tmk*3 genes (Δ*atf*1, Δ*tmk*3). The WT, Δ*atf1* and Δ*tmk*3 strains were treated with a blue light pulse (1200 µmol/$m^2$) or keeping in darkness. Total RNA was extracted from each sample using Trizol protocol. Photoinductions were confirmed analyzing expression of *blu*1, *grg*2, *env*1, and *blu*4 (dependent of blue light) by end-point RT-PCR. The transcriptome were obtained by the NextSeq 500 High Output kit of illumina, the output was up to 400 million of reads (1x75b). Results from RT-PCR proved that Tmk3-Atf1 pathway is related to regulation of some genes dependent of light. Sequence quality was analyzed employing FASTQC and the reads were mapped versus the *T. atroviride* genome. The differential expression analysis is in progress to know how Tmk3 and Atf1 participate in blue light-dependent gene regulation. Accordingly, our results indicates that the Atf1 function is both dependent and independent of Tmk3 signaling pathway on blue-light regulation.

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# Assessing the level of expression of mir 132 and 203, and the methylation status of the promoter region of this miRNAs in cell lines of breast cancer and their role as posttranscriptional regulators repair genes DNA damage BRCA-1, BRCA -2 and ATM

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Breast cancer is one of the most commonly diagnosed cancer types in women worldwide, 1.3 million new cases each year and a total of 450 000 deaths worldwide are estimated. In Mexico, breast cancer ranks first among female cancer patients in incidence and mortality. BRCA-1, BRCA-2 and ATM are tumor suppressor genes that encode proteins involved in DNA repair. Defects in these genes can result in malfunctioning proteins, thus DNA damage cannot be repaired properly. Mutations in BRCA-1, BRCA-2 and ATM, and posttranscriptional and epigenetic deregulation of them leads to increased risk of developing cancer. As a result of deregulation, these genes can be expressed on tumor cells and provide a high-strength, quasi-immediate repair of the DNA damage induced by anti-tumor therapies. MiRNAs are endogenous 22-nucleotide-long RNAs that bind to the 3'UTR region of mature mRNAs; microRNA expression may in turn be regulated by epigenetic factors such as methylation. Increased methylation of the promoter region of a gene can lead to the reduction of its expression, while increased methylation of the transcribed region may have a variable effect on gene expression. This work aimed to establish the methylation status of the miR-132 and miR-203 promoters, and assess their expression level in four breast cancer-derived cell lines. The expression level was assessed through TaqMan qPCR and the methylation analysis was performed using EZ DNA Methylation Kit-startup. Through these analyzes we found differential methylation status of the promoter regions of miR-132 and -203 among the cell lines. On the other hand, miR-203 expression level was decreased, while mir-132 was over expressed on the four breast cancer-derived cell lines. Experiments to measure DNA damage repair are being carried out. This data will shed light on the role of miRNA regulation in DNA repair mechanisms

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# Hyperglycemic effect on proliferation and apoptosis of human umbilical cord mesenchymal stem cells from children of normoglycemic and diabetic mothers.

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Keywords: Diabetes, Hyperglycemia, Cytotoxicity, Proliferation, Apoptosis, Wharton's jelly cells

**Introduction.** Diabetes is mainly characterized by high blood glucose concentration, triggering serious diseases. World Health Organization data report that diabetes prevalence in México is around 10.4%, being women at reproductive age highly affected (1). Children of diabetic mothers have more risk of perinatal morbidity and mortality. Maternal pregestational diabetes (type 1 or type 2) have an increased risk for broad-spectrum birth defects (2). The congenital malformations associated with diabetes can affect several organs or systems, but neural tube defects (NTDs) and cardio-vascular defects, are the most common (3). Hyperglycemia is a mechanism that generates oxidative stress through the induction of hypoxia when mitochondrial activity increases. Hyperglycemia alters signaling pathways and expression of proapoptotic genes, which has been proposed as factors of birth defects. The study of hyperglycemia in fetal development and its effects has been done in animal models, *in vivo* in mice and rats and *in vitro* in neural stem cells (NSC) and neural crest cells from rodents (4). Since the neural tube is derived from NSC, it is hypothesized that in diabetic pregnancy NTD results from altered expression of developmental control genes, leading to abnormal proliferation and cell-fate choice of NSC. In particular, maternal diabetes inhibits expression of *Pax3*, a transcription factor that is expressed in neural crest and neuroepithelial cells. As a result, cardiac neural crest and neuroepithelial cells undergo apoptosis by a process dependent on p53 protein. This provides a cellular explanation for the cardio-vascular defects and NTD induced by diabetes in pregnancy. On the other hand, there are no reports in literature about a cell-based model for the study of maternal diabetes during pregnancy in humans. We propose the Wharton jelly mesenchymal stem cells (hWJMSC) as a human cellular model, because they can be easily isolated from umbilical cords of children of normoglycemic or diabetic mothers, without risks for the donor or ethical concerns. In addition, hWJMSC represent a more primitive population than their adult counterparts, they also retain characteristics of primitive stem cells, like the expression of embryonic stem cell markers and multipotent differentiation capacity (5). For these reasons, hWJMSC could provide an attractive alternative cell model to study the mechanisms behind diabetes and pregnancy.

**Methods.** The hWJMSC where isolated from human umbilical cord matrix of Children of Non-Diabetic Mothers (CNDM) and from Children of Diabetic Mothers (CDM). The matrix mesenchymal stem cells were purified by FACS with negative selection of endothelial and/or hematopoietic CD31, CD34 and CD45 markers. The isolated cells were proliferated and characterized for the expression of CD13+, CD31-, CD34-, CD44+,

CD45-, CD73+, CD90+ and CD105+ by flow cytometry. Mesenchymal stem cells were differentiated into neuron-like cells and adipocytes. The adipocyte differentiation was confirmed by Oil Red O staining (Sigma-Aldrich), while the neuron-like differentiation was assessed by the expression of neuron-cell markers β-III Tubulin, MAP2 and Nestin by RT-PCR and indirect immunofluorescence. The hWJMSC were proliferated in different concentrations of glucose (control, 20, 30 and 40 mM D-Glucose) in two culture medium: Chang and DMEM+10% FBS. To determine the doubling time, proliferation curves were seeding at $2x10^4$ cells and counting every 24 hrs. For apoptotic assays, 1-2 $x10^5$ cells were seeded in CHANG medium under the following conditions: control (0h), 2h and 24h with 40mM D-glucose. Samples were processed with Annexin V-FITC apoptosis detection kit (eBioscience) and analyzed by flow cytometry. Finally, the expression of pro-apoptotic and oxidative stress genes BNIP3, p53, HIF1α and AMPK were assessed at 0, 1, 2, 5, 24 and 48h of treatment with D-glucose, by semi-quantitative RT-PCR.

**Results.** The growth curves showed an exponential proliferation trend. The doubling time was determined for control 20, 30 and 40 mM D-glu in each medium. From CNDM in Chang medium was 22.8 ± 2.8 in control conditions, and increase to 24 ± 3.2, 26.9 ± 3.5 and 34.0 ± 7.1 at 20, 30 and 40 mM D-glu respectively. In DMEM was 43 ± 12.2 in control conditions, and increase to 57.6 ± 16.2, 66.8 ± 25 and 80.2 ± 34.3. Meanwhile for CDM in Chang was 24.2 ± 3.2 in control conditions, at 20mM D-glu was 23.1 ± 1.9, and increase to 26.5 ± 3.4 and 30.5 ± 6.2 at 30 and 40mM D-glu respectively. For DMEM was 55 ± 33 in control conditions, and 61.2 ± 25.2 and 77.5 ± 31.5 at 30 and 40mM D-glu. In apoptosis assays, in control conditions of CDM, the 35% of cells showed positive apoptosis, while at 2 and 24h after treatment with 40mM D-glu, apoptotic cells increases to 60% and 45%, respectively. Relative expression of genes tends to increase respect to control. HIF1α rises 200%, while BNIP3, p53 and AMPK to 400%, over control, being at 1h the smallest increase but as time progressed to 2, 5, 24 and 48h the expression enhance.

**Discussion.** The doubling time increased in cells from NDCM and DCM according to the increment in glucose concentration, in both growth mediums. The percentage of apoptotic cells increased approximately 20% in cells cultured in 40mM D-glu over control as well as the expression of BNIP3, p53, HIF1α and AMPK increased in hyperglycemic conditions relative to control. Taken together, our results suggest that hyperglycemia has a pro-apoptotic effect on hWJMSC.

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# Biological activation of hypoxia responsive elements in the transcriptional regulation of the CYP2B6 in meduloblastoma cells

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**Background**. Cytochrome P450 (*CYP*) is a superfamily of genes that codify for a wide group of hemo-thyolated enzymes, which catalyzes oxide-reduction reactions for most of the chemical agents used in cancer therapy. The hypoxia and activation of the hypoxia inducible factors (HIFs) play a critical role for therapy resistance in tumors; however, their contribution into modulate CYP expression and consequences in drug resistance are not clearly understood. Recently, we have described that *in vitro*, hypoxia increases resistance to cyclophosphamide, ifosfamide and vincristine in meduloblastoma cells, which is related with the modulation of the CYP2B6 expression. The aim of the present work was to determine the biological functionality of two upstream hypoxia responsive elements in the *CYP2B6* human gene.
**Methods**: Monolayer medulloblastoma Daoy cell cultures were incubated in normoxia and hypoxia (1% and 0.1%) for 4, 8 and 24 h. Protein levels and mRNA of HIF-1 alpha and CYP2B6 were determined by Western blotting and RT-qPCR, respectively; whereas, carbonic anhydrase IX (CA-IX) protein levels were considered as indicator of the HIF-1 alpha pathway activation. Prediction of the hypoxia response element core **5´-RCGTG-3´** (HRE) was performed with noncoding region -2,000 to +1 pb for alignments with CLUSTAL2W and JASPAR database (http://jaspar.genereg.net). The upstream segment was cloned from human genomic DNA in a vector bearing a reporter gene; response to hypoxia was analyzed by determination of luciferase activity.
**Results**: Protein levels of CYP2B6 were significantly augmented in hypoxia 0.1% at 4 and 8 h comparing to normoxia; however, decrease at 24 h. In contrast, there was no change in the expression of CYP2B6 with hypoxia 1% at 4 and 8 h, but it also decrease at 24 h. The CYP2B6 upstream region bearing HRE elements was amplified by PCR and cloned in a vector with a reporter gene (luciferase) and sequenced for confirmation. The luciferase activity increases in hypoxia (0.1% oxygen) at 4h, and decrease at 24 h as compared with normoxia. In contrast, hypoxia at 1% oxygen did no affects the luciferase activity at 4 h, and also diminished at 24 h ($p<0.0001$).
**Conclusions**: Hypoxia regulates the expression of CYP2B6 protein in Daoy medulloblastoma cell line and it is dependent of time of exposition and oxygen concentration. The upstream CYP2B6 cloned shown reporter gene activity under hypoxia 0.1% oxygen but not at 1% oxygen. These results support the hypothesis of biological activity of the HRE enhancers in the CYP2B6 gene in response to hypoxia.

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# Identification of genes involved in the pathogenicity of the plant pathogenic fungus *Macrophomina phaseolina* in different hosts

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*Macrophomina phaseolina* is a necrotrophic fungal pathogen which attacks a wide range variety of plant species, including maize (*Zea mays*), common bean (*Phaseolus vulgaris*), wheat (*Triticum sp.*), among others. However, the study of their molecular bases of the pathogenic process is still in nascent state, as well as the knowledge of the genes involved in the establishment of the disease in the hosts. In this study, we identified from expressed sequences tags (ESTs), several genes involved in the pathogenicity of *M. phaseolina* in two different hosts: *Arabidopsis thaliana* and *Phaseolus vulgaris*.

We created three data bases corresponds to the full transcriptome sequences of *M. phaseolina, A. thaliana,* and *P. vulgaris.* Using Blastn against to the three data bases previously created, we characterized 101 and 78 ESTs from an interaction between *Macrophomina phaseolina-Arabidopsis thaliana* and *Macrophomina phaseolina-Phaseolus vulgaris*, respectively. By means of Blastn, Blastp and Jpred we identified the ESTs that showed similarity with hypothetic or unknown proteins.

A total of 25 and 10 genes of *M. phaseolina* were identified in the interaction with *A. thaliana* and *P. vulgaris*, respectively. The *cipC* gene that encode to a toxin with antibacterial proprieties was found in both interactions. In the interaction of *M. phaseolina* with *P. vulgaris*, we found an N-cianovirin encoding gene, a toxin with antiviral activity reported in several fungal pathogens as *Magnaporthe oryzae.* Furthermore, in the interaction *M. phaseolina* with *A. thaliana,* we identified a polyketide synthase encoding gene, involved in the synthesis of polyketide T-toxin. This result suggested that *M. phaseolina* use specific toxins to infect different hosts. In addition, we identified an endo-1, 4-beta-xilanasa coding gene, homolog to gene *Xyn11A* of *Botrytis cinerea,* involved in the host cell wall degradation, among other genes.

This study represents an approach in the elucidation of the molecular basis of pathogenicity of this fungal pathogen.

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# Evolutionary history of the histone acetyltransferase Gcn5

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Acetylation of specific lysine residues of histones is a remodeling chromatin mechanism associated to transcription activation, which is realized by histone acetyltransferases. Gcn5 is the catalytic subunit of the main histone acetylation complex. In pathogenic fungi, Gcn5p is involved in the genes induction related with stress, filamentous growth, differentiation cellular process and virulence. The analysis of evolutionary history of the Gcn5 has indicated that the enzyme is highly conserved in fungi from its ancestral forms. Additionally, this analysis suggested that duplication of Gcn5-encoding genes may have occurred in a recently time of manner specific in several species. However, there is no evidence of the evolutionary history focused on biological and molecular process. For this reason, we reconstruct the Gcn5 evolutionary history in a several eukaryotic organisms, focused on fungi.

Using Mega7 program we reconstructed an organism's time tree and a Gcn5p phylogenetic time tree using the small subunit ribosomal RNA (SSU rRNA) and the Gcn5p amino acid sequences, respectively. We selected several species of the kingdoms: fungi (52), Protista (8) and plantae (3). Blastp was used for obtain the number of Gcn5p copies in the proteome of the species selected. The motifs in Gcn5p sequences for each phylum were determined with the program Skylign.

The divergence times in each phylum of the Gcn5p phylogenetic time tree match with the divergence times and evolution of the species time tree. The protozoa *Theileria annulata* was the most distant ancestor with one copy of *gcn5*. A few of this most distant organisms (Protozoa, amoebozoa and Zygomycetes) had more than one *gcn5* copy. In the fungi kingdom, the phyla of basidiomycetes have only one copy of the *gcn5*. Moreover, the ascomycetes showed a peculiar gene duplication event in some organisms, particularity in the pathogens. Several species as *Arthroderma benhamiae, Metarhizium acridum, Ophiostoma piceae, Magnaporthe oryzae, Sporothrix schenckii, Fusarium oxysporum, Aspergillus niger, Histoplasma capsulatum, Microsporum gypseum, Blastomyces dermatitidis,* and *Trichophyton rubrum* obtains a second copy (three in the case of *F. oxysporum)* of *gcn5* in a time of divergence relatively close. In addition, the comparison of the residues with catalytic activity in each copy of this species indicated that the copies preserve the molecular function. Suggesting a model of concerted evolution in this group.

Our results indicated that the histone acetyltransferase Gcn5 has had a similar evolution to that of the species.

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# Role of translation initiation factors eIF4E and eIFiso4E during cold stress response in *Arabidopsis thaliana.*

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Plant abiotic stress response is a complex process that occurs when plants are exposed to unfavorable conditions during growth, such as high or low temperature, drought, elevated ground salinity and others. This process involves a specific gene expression patterns as well as tight regulation of protein synthesis to increase plant resistance and survival capacity. Translation regulation emerges as important step in stress response. There is evidence of general translation inhibition under stress, but particular protein production remains active for homeostasis maintenance. The mechanisms involved in translation specificity under stress are poorly understood. In our group we focus on the role of translation initiation factors belonging to the 4E family (eIF4E) in *Arabidopsis thaliana* response to freezing temperature. These factors are major players in protein synthesis regulation in other organisms. They recognize the 5' end cap structure on mRNAs and recruit the rest of the translational machinery. In *Arabidopsis* there are two isoforms termed eIF4E-1 and eIF4E-2 or eIFiso4E that under normal growth conditions apparently have redundant functions. However, experimental evidence pinpointed certain isoform preference in selection of mRNAs encoding stress response proteins. Whether there is specialization of these factors in abiotic response as reported for other organisms remains an open question. Here we tested the expression of these factors after cold treatment and found that their production is upregulated under this condition. On the other hand, phenotypic analyses of plants overexpressing either eIF4E (*35S:eIF4E*) or eIFiso4E (*35S:eIFiso4E*) compared with wild type plants showed increased resistance after a freezing treatment. Interestingly, the absence of eIFiso4E (*AteIFiso4E-1*) has a detrimental effect on plant response to this type of stress even in the presence of eIF4E suggesting that the role of these isoforms in freezing tolerance is not completely redundant. In order to investigate at molecular level the specialized action of this factors during stress response, we have selected a group of cold activated genes proposed as preferentially translated by each one of these factors to analyze their expression pattern in the mutant lines mentioned before. We hypothesize that selective translation of stress responsive mRNAs by eIF4E isoforms might underlay their specialized function in cold temperature plant response.

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# Functional Analysis of miR-196b and its Role in Breast Cancer Radioresistance

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**Background:** Understanding the molecular mechanisms responsible for sensitizing radioresistant breast tumors to ionizing radiation (IR) treatments is clinically relevant. Recently, microRNAs (miRNAs) have emerged as potential therapeutic agents able to modify cellular sensitivities to IR treatments through their post-transcriptional regulation of IR-activated pathways. The miRNA miR-196b, a known regulator of HOX genes, was previously identified by our group as a miRNA down-regulated in a subpopulation of radioresistant MCF-7 breast cancer cells.

**Results:** Here, we determined the expression of miR-196b in the breast cancer cell lines T47D, MCF-7, MDA-MB-231, and the epithelial cell line MCF10A using RT-PCR. We found that the triple negative breast cancer cell line MDA-MB-231 exhibits low levels of miR-196b expression when compared to estrogen receptor-positive cell lines and non-tumorigenic MCF10A cells; which correlates with its known radioresistance. We also found that exposure of breast cancer cell lines to various dosis of IR (0, 2, 4, 6, 8 and 10 Gy) affected miR-196b expression. Moreover, ectopic expression of miR-196b in MDA-MB-231 cells, achieved by transfecting a miR-196b precursor (10nM), sensitized these cells to IR as measured by survival MTT assays and clonogenic assays. Through bioinformatic analysis, we found that SMAC3, LIN28B, WNT2B, CDC7 and DCLRE1C are predicted targets of miR-196b. Further studies are in progress to determine if these targets play a role in the biological responses elicited by miR-196b in tumor cells. Our results suggest that miR-196b reconstitution could be used as a therapeutic strategy aimed at sensitizing tumors to IR in breast cancer.

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# Involvement of Pygopus2 on the regulation of migration and invasion capabilities of cervical cancer cell lines

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Cervical cancer is the second leading cause of death in mexican women, it has been associated with human papillomavirus infections since viral oncoproteins affect the expression of proteins related to cell cycle progression, differentiation, tissue homeostasis, cellular motility and evasion of apoptosis. One of these deregulated pathways is the Wnt signalling pathway, which plays an important role in cancer development, establishment, and expansion. The aim of this study was to assess the expression profile of different members of the Wnt pathway at the mRNA and protein levels in four cervical cell lines with different origins. RNA expression data was obtained through qRT-PCR, and protein expression data trough western blot. We found high mRNA levels on 9 out of 14 evaluated components in CaSki cells, this suggests that this cell line has the highest activity of this signalling pathway among the other three cell lines. Wnt signalling is apparently less active in SiHa where we found the lowest mRNA expression of the evaluated components. At the protein level, the cervical cell lines had a very similar pattern with the exception of the nuclear proteins Pygo2 and ß-catenin, which had and opposite pattern of expression. This is interesting because these crucial members of the Wnt pathway are supposed to work together, though their expression profiles show they might not. We inhibited Pygopus2 through stable expression of short-hairpin RNA and found its impact on cell proliferation and migration. Our results show that the Wnt pathway is a promising therapeutic approach for human cancers such as cervical cancer, colorectal cancer, and lung cancer, among others which share genomic alterations of this pathway.

# Design and construction of an inducible system locus-directed to produce DSB in *Giardia duodenalis*

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*Giardia duodenalis* is a flagellated protozoan parasite that commonly causes diarrheal diseases, named giardiasis. During its life cycle, several rounds of DNA replication, cytokinesis and karyokinesis occur; therefore, the parasite is in constant flux in ploidy, and DNA repair is crucial for maintaining the integrity of its genome. During Giardia life cycle, its genome can suffer ruptures due to endogenous or exogenous factors, and this damage can be repaired by different mechanisms. The most severe lesion is the DNA double-stranded break (DSB) since one single DSB not repaired may induce apoptosis.

The DSB´s also are introduced naturally during meiosis process, in order to generate genetic diversity by homologous recombination and into eukaryotic cells may be produced by the programed expression of specific endonucleases. Meganucleases are highly specific DNA cleaving enzymes that are encoded within genomes of all forms of microbial life as in eukaryotic mitochondria and chloroplasts. A hallmark of the meganucleases is the contrast between their small size (200 aa) and their long DNA target sites (>20 pb).

In this work, the meganuclease I-SceI target site (TAGGGATAACAGGGTAAT) was introduced into the Giardia genome through homologous recombination (creating I-SceI target site transgenic) by its previous cloning into an integrable vector along with flaking VSP160 regions to promote recombination; the plasmid was introduced by electroporation and transfected cells were selected using puromycin. The construct was verified by PCR analysis. Separately, I-SceI nuclease was cloned in an inducible gene expression system containing NLS (nuclear localization signal) and an epitope MYC in order to monitor protein expression. Cells were transfected by electroporation and selected by geneticin. Plasmid presence was confirmed by PCR analysis with specific primers to I-SceI. After transfection, I-SceI expression was induced (doxicycline1 µg/mL, 7 hours) and its nuclear localization was confirmed by immunofluorescence.

With this system we would be able to stablish a locus-directed DNA double-stranted cleavage system in order to study recruitment of factors involved in DNA repair process and chromatin remodeling during this process.

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# Twin, a Component of the CCR4-NOT Complex, is involved in Thermal Nociception in *Drosophila*

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Abstract:

The animal capacity for the detection of potentially damaging stimulus is called nociception. If the system is to be capable of integrating sensory information such as nociception and elicit a correct behavioral response, an extremely complex network of cellular and molecular machinery must work in exquisite coordination. *Drosophila melanogaster* has proven to be an excellent model for the identification and characterization of genes involved in sensory perception and nociception (Tracey, Wilson, Laurent, & Benzer, 2003) (Neely et al., 2010).

In this work we demonstrate that *twin* loss of function is sufficient to reduce thermal nociception and cause synaptic hypertrophy. This gene codes for a deadenylase and is part of the CCR4-NOT complex that is involved in translational control of gene expression (Morris, Hong, Lilly, & Lehmann, 2005). Furthermore, using an interactome analysis approach we propose that the Twin and the CCR4-NOT complex interact with transcripts of other genes also involved in nociception via the "AU rich elements" (AREs) most likely regulating their translational levels by deadenylation (Temme, Simonelig, & Wahle, 2014). Finally we propose an integrative model for *twin's* regulatory function in nociception.

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# Transcriptional profiling of the female reproductive tract differentiation process in mice

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Congenital absence of uterus and vagina is a relatively frequent event (1:4500 women) and its etiology, along with other Müllerian duct (MD) anomalies, is unknown. The molecular basis of the female reproductive tract (FRT) morphogenesis, including its gene regulatory networks, has not been studied in depth. Lhx1, Hox and several Wnt proteins participate in the formation of MD. Once elongated, differentiation of female MD starts at embryonic day E13 and concludes in E17, when complete uterine, fallopian and vaginal structures are displayed, and Wolffian male ducts regressed. In order to identify genes potentially involved in the morphogenesis of the FRT, in this work we characterised the transcriptome of CD-1 murine mesonephric tissues harbouring Müllerian and Wolffian ducts from E13 to E17. Differential expression patterns between age and gender were observed, and transcript signatures corresponding to both female background and female-specific temporal activation were identified. The Wnt (p=7.8e-05) and TGF-Beta (p=7.1e-05) pathways, *serine peptidase inhibition* (p=4e-05), and *olfactory receptor activation* (p=4e-05) functional terms were overrepresented in the latter. Common ontologies such as *receptors coupled to G-proteins* and *surface receptor signal transduction* were identified in both males and females, however, these were constituted by different agents. These data will contribute to the current knowledge on the gene regulatory networks of the FRT differentiation. Finally, by combining our results with a database of human deletions and the identification of genes potentially regulated by Lhx1, we propose a strategy for prioritization of candidate genes for MD anomalies. CONACYT-133273, UNAM-PAPIIT IN219912.

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# Cellular localization of 5S rRNA genes and transcripts in the parasite *Leishmania major*

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5S ribosomal RNA (rRNA) is an essential component of the large ribosomal subunit, where it links together and coordinates all of the functional centers of the ribosome. 5S rRNA also regulates the signaling pathways that couple cell proliferation with ribosome production. In eukaryotic cells, 5S rRNA is synthesized by RNA polymerase III (Pol III). Most organisms contain from 100 to 1000 copies of the 5S rRNA genes that are organized into tandem arrays. However, the genome of the protozoan parasite *Leishmania major* contains only 11 copies of the 5S rRNA gene, which are interspersed and associated with other Pol III-transcribed genes. By performing *in silico* analyses, we showed that, in general, the number and order of the 5S rRNA genes is conserved between different species of *Leishmania*. All 5S rRNA genes contain the putative internal promoter regions: boxes A and C and the intermediate element. These regions are necessary in other eukaryotes for the binding of transcription factors TFIIIA, TFIIIC and TFIIIB, which allow Pol III transcription. Interestingly, only TFIIIB has been identified in *L. major*. By 5´-RACE experiments we showed that transcription of the 5S rRNA genes starts at a G that corresponds to the first nucleotide of the gene. Thus, processing in the 5' region is not required to generate the mature 5S rRNA. In contrast, 3´-RACE assays demonstrated that Pol III transcription ends in a tract of Ts localized downstream of all the 5S rRNA genes, showing that processing is needed in the 3´ region of the 5S rRNA. While in most organisms 5S rRNA genes are normally associated with the nucleolus, combined FISH and indirect immunofluorescence experiments showed that 5S rRNA genes are dispersed throughout the nucleus in *L. major*. In contrast, 5S rRNA transcripts were localized within the nucleolus, and scattered throughout the cytoplasm, where mature ribosomes are located.

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# Molecular characterization of the effect of ovarian-specific transcription factors on the activity of *Fbxw15* promoter.

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El presente trabajo se llevó a cabo en las siguientes Instituciones
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One of the most important processes to maintain the reproductive potential in mammals is folliculogenesis which comprises, conservation, viability and maturation of primordial germ cells (CPGs) for the subsequent settlement of female gametes or ova. This process requires a precise molecular coordination driven by a series of genetic interactions between somatic and germ cells; deficiency or bad signaling in such interactions can cause abnormal or atretic follicular formation. Derived from a microarray analysis, our group previously showed the biphasic expression of a gene called *Fbwx15* in murine oocytes, such biphasic expression coincides with the onset of follicular assembly and the further follicular development independent of gonadotropic stimulus. This gene encodes a protein with an box F motif (Fbxw15); which specifically interacts with Skp1 and Cullin forming the SCF-Fbwx15 complex (Skp1-Cullin-F-box complex) to regulate the proteolysis of HBO1 (Complex Origin and Recognition of Union to histone acetyltransferase) via the ubiquitin-proteasome system, thus mediating cell proliferation. In this study the promoter region of *Fbxw15* was analyzed *in silico* and binding sites for transcription factors related to various cellular processes such as proliferation, cell differentiation (MybL2, Nf1f, Ap1), regulation of histones (Oct1) and specific to folliculogenesis (Foxl1, Foxp1, Hoxa3, Dlxf), were determined. These binding sites were evaluated by generating sequential deletions of the parental plasmid (pRL/Fbxw15) containing such sites and by analyzing the luciferase transcriptional activity in a heterologous system. The obtained sequential constructs were transfected into Hamster Ovary Cells (CHO-K1). The results showed that by eliminating binding sites for DLXF and Hoxa3, luciferase activity significantly decreased in 86% compared to the one observed in the parental plasmid. Interestingly the elimination of NF1 and Ap1 sites which are close to the TATA box showed no significant difference in transcriptional activity, this is probably because a binding site for CEBP was identified several base pairs downstream of the TATA site. The latter may suggest that Dlxf, Hoxa3 and CEBP interaction could be important in regulating the transcriptional activity of *Fbxw15*.

# Deciphering the Role of H3K9 Methylation in Plant Embryo Development

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Histone modifications constitute one of the major epigenetic mechanisms involve in the control of transcription. Covalent modifications such as methylation, acetylation, phosphorylation and ubiquitination are responsible for the different states of chromatin, and therefore constitute important factors that control gene expression. Lysine methylation at histone 3 (H3K9me) is a well studied chromatin mark in animal and plant models. Its function is generally linked to transcriptional silencing by heterochromatin formation. It has been demonstrated that gene silencing caused by H3K9me activity strongly affect diverse developmental processes. Indeed this covalent modification is essential for embryo development in mammals (Tachibana et al., Genes & Dev, 2002).

Genetic studies of Arabidopsis have discovered numerous transcription factors, signaling cascades and hormone pathways that control pattern formation and morphogenesis in early embryos. Hence during the different stages of embryo development, a complex network of transcriptional programs are activated or repressed in a dynamic cross talk. In plants, H3K9me2 has been mainly related with transposon silencing and other repetitive sequences, most of the time acting in a combinatory function with DNA methylation at CHG contexts. Here, we used cytological and molecular approaches to determine the temporal and spatial expression pattern of H3K9me2 activity during embryogenesis. Our functional analysis demonstrated that histone methyltransferase genes in Arabidopsis (SUVH genes) are transiently required for normal embryo pattern formation in early embryogenesis, with some genes playing a more important role than others. Additionally, specific RNA-seq analysis raised the possibility that H3K9me2 could be acting as a transcriptional regulator of specific loci that are involved in development. Accordingly, RNAseq data suggests that large number of embryo patterning genes are direct or indirect targets of SUVH genes. Our results suggest that H3K9 methylation is an epigenetic mark involved in early embryo patterning and seed development.

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# Construction of a CRISPR/Cas9 system for gene editing in *Giardia duodenalis*

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*Giardia duodenalis* is a Protozoan, binuclear and flagellated organism that causes giardiasis and considered an early divergent organism. Giardiasis is a recurrent disease mainly in children in developing countries, making it important for its study. Lately, there have been several molecular approaches aimed to discern molecular mechanisms of different processes, however the role of many genes involved in these is still largely unknown. For this reason, it is necessary to implement new molecular biology tools as gene editing. Nowadays, there are effective editing mechanisms, which has been shown to yield good results and them easy to use such as the CRISPR/Cas9 system. This system is based in a nuclease Cas9 and a gRNA that guides the nuclease Cas9 to their target sequence to generate a double-strand break (DSB) in DNA. In this work, we have designed and implemented this system in *Giardia*, by constructing an optimizing and inducible plasmid for the expression of both the gRNA and nuclease Cas9 in *Giardia*. We cloned, and confirmed the expression of the nuclease by Western-blot and the expression of the gRNA by RT-PCR in transfected trophozoites of *Giardia duodenalis*. In addition, to direct Cas9 to the nucleus, we added NLS's (nuclear localization sequences), this was confirmed by immunofluorescence. Also, we determined the optimal concentration of doxycycline to induce Cas9 nuclease. Finally, DSB caused by Cas9 was determined and confirmed by PCR with primers flanking the DSB In conclusion, it is possible to use this system in *Giardia* and begin to implement it as a molecular tool for gene editing.

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# The role of MicroRNAs in *Marchantia polymorpha* during water deficit

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The bryophyte *Marchantia polymorpha* is a non-vascular plant with a dominant haploid life cycle. This group is considered as the earliest divergent group of land plants, offering a unique window into plant evolution. MicroRNAs are small non-coding RNAs that participate in diverse processes including plant stress responses in vascular plants, but nothing is known about their participation during stress in non-vascular plants. The aim of this work is to characterize the response of *M. polymorpha* to NaCl exposure; and additionally to address the role of new and conserved microRNAs in response to salt stress in this basal plant.

*M. polymotpha* thalli exposed to 150 and 250 mM of NaCl showed morphological defects, and osmolyte accumulation during 24 hour of exposition. Plants treated lost around 60% of their fresh weight during 24 hours of exposition to salt stress. The group exposed to 150 mM showed no change in the osmolyte accumulation but the group exposed to 250 mM showed a rise in the osmolyte concentration. Interestingly, the photosynthetic rate was not disrupted in the first group, while the exposition to 250 mM NaCl drastically affected photosynthesis.

Due to the known role of abscisic acid (ABA) during abiotic stress in vascular plants, we also investigated the effect of this hormone applied exogenously to *M. polymorpha* during 24 hours. Exposition of thalli to 1, 5 and 10 µM of ABA did not change the morphology of the *Marchantia* thallus or affected its photosynthetic rate, nevertheless, we observed a protective effect of this hormone in plants pretreated with ABA upon NaCl exposure.

We performed high throughput sequencing of small RNAs analysis of thalli exposed to 150 or 250 mM of NaCl to identify microRNAs responding to salt stress. Preliminary results showed that accumulation of most of them was reduced during NaCl stress. We confirmed the accumulation of conserved microRNAs during optimal conditions using an RT-qPCR strategy. Current work is aimed at characterizing the accumulation of conserved and novel microRNAs during NaCl stress and ABA treatment and further analysis of their involvement in stress responses, including identification of their regulatory target mRNAs.

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# Identification of production of alkyl-quinolones molecules by *Pseudomonas aeruginosa* strains with an atypical quorum sensing system

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**Introduction.** *P. aeruginosa* controls many virulence factors by using two quorum-sensing systems, *las* and *rhl*. The *las* system is composed of the LasR regulator protein and its cell-to-cell signal, *N*-3-oxododecanoyl homoserine lactone, while the *rhl* system is composed of the RhlR protein and the signal molecule *N*-butanoyl homoserine lactone. Additionally, a third quorum-sensing system based in alkyl-quinoone molecules (AQs) has been reported in *P. aeruginosa*, the *Pseudomonas* quinolone signal (PQS) 2-heptyl-3-hydroxy-4-quinolone), whose precursor is 2-heptyl-4-quinolone (HHQ), both molecules are signals of this system which is involved in the production of pyocyanin. The *pqsABCDE*, *pqsH* and *pqsL* genes are responsible for the synthesis of AQs, The PqsR transcriptional regulator in turn, upon binding with HHQ or PQS, activates the transcription of the *pqsABCD* operon. The transcription of *pqsR* gene is positively regulated by LasR and negatively regulated by RhlR (Wade *et al.* 2005). However, there are strains with defects in the key elements of the *las* system such as the case of the strains 148, ID4365 and INP43 of *P. aeruginosa* (Grosso-Becerra *et al.* 2014; García-Contreras *et al.*2015). Thus, to know how these strains with an atypical quorum sensing system are able to produce PQS in the absence of the *las* system, is the main approach of this research project related with the regulation of the AQs synthesis and pyocyanin production. **Objective:** To determine whether the strains 148, ID4365 and INP43, with a defective *las* system, are able to produce AQs. **Methodology:** Identification of PQS production. In order to identify AQs production, a biosensor (*P. aeruginosa* PAO1ΔpqsA with a transcriptional fusion of *pqsA'::lux*) and supernatants from strains of study were used as reported by Diggle *et al*., 2011. **Results.** Figure 1 shows that 148, ID4365 and INP43 strains are able to produce the AQs molecules, even though these strains have defects in the *las* system. This shows the atypical behavior of these three strains compared to that reported in quorum sensing system of the reference strain *P. aeruginosa PAO1.* The bioluminescence generated by biosensor strain was reported as Relative light units (RLU)/optical density (D.O).

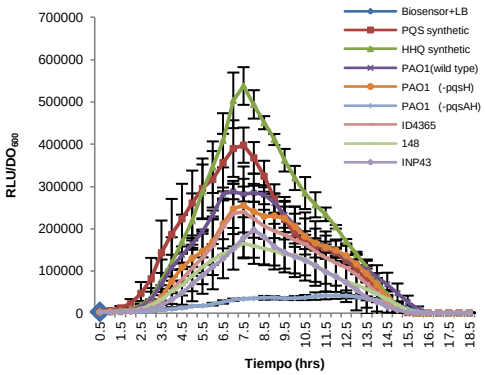


**Figure 1.** Biosensor assay for AQs detection.

**Conclusion.** Strains of *P. aeruginosa* 148, ID4365 and INP43 are capable of producing AQs even with defects in the *las* system, a fact that makes them in atypical model of quorum sensing system with biomedical importance.

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# MicroRNAs profiling identify miR-143 as a novel predictor of pathological complete response to chemotherapy in triple negative breast cancer

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**Background:** Triple negative breast cancer (TNBC) patient's exhibits resistance to chemotherapy and poor survival rates. Distinct therapeutic combinations based in anthracyclines and taxanes have been used to reduce high mortality. Recently, novel regimens of neoadjuvant chemotherapy for locally advanced TNBC have achieved pathological complete response (pCR) rates of 10-50% cases. Evaluation of pCR during oncologic treatment is decisive to identify those patients that response or not response to neoadjuvant chemotherapy. MicroRNAs (miRNAs) are small non-coding RNAs that represent novel and potential predictive biomarkers useful to identify the patients who will get pCR in cancer.

**Results:** In this study, we profiled 754 miRNAs using TaqMan Low Density Arrays, and we identified a signature of 11 miRNAs differentially expressed in TNBC tumors from patients that achieved a pCR after fluorouracil, adriamycin, cyclophosphamide /cisplatin/ paclitaxel (FAC--CDDP/paclitaxel) regimen as neoadjuvant treatment. Eight miRNAs (miR-9-3p, miR-30a-3p, miR-135b, miR-135b*, miR-380-5p, miR-941, miR-652, miR-181c*) were overexpressed and three (miR-770-5p, miR-584, miR-143) were downregulated. Bioinformatics analysis revealed that majority of gene targets of miRNAs deregulated in pCR group were involved in diverse cellular processes altered during tumorigenesis mainly in immune response. Particularly, miR-143 was downregulated in pCR group and it may be involved in the negative regulation of proliferation of T and B cells. Interestingly, miR-143 potentially targets Programmed Cell Death 1 (PDCD1), a gene involved in the inhibition of immune response against the tumors. Interestingly, TNBC patients can achieve a pCR in part by lymphocytic infiltration to stroma of tumors before treatment. MiR-143 may prevent the proliferation of Th lymphocytes, which are activated by neoantigens released for tumor cells after chemotherapy. Downregulation of miR-143 was confirmed in an independent cohort of patients with pCR and four breast cancer cell lines. Association of miR-143 expression with survival rates could stablish that miR-143 may be a prognosis biomarker to FAC--CDDP/paclitaxel response. Moreover, restoring expression of miR143 will enable inhibit its target PDCD1 and subsequently to increase the immune response for eliminate tumor cells *in vitro*. Functional studies in progress will define if miR-413 may target PDCD1 and modulate the immune response against the tumors. Thus, miR-143 could also represent a therapeutic and survival prognostic biomarker of pCR in TNBC.

**Key words:** Triple negative breast cancer, neoadjuvant chemotherapy, pathological complete response, immune response and biomarker.**Acknowledgement:** CONACY (Salud-233370), INCAN, FUCAM, UACM

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# A comparative analysis of perinatal disorders and DNA methylome profiling at early childhood: implementation of a pilot study

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One in five women suffer complications during pregnancy in Mexico. The main causes of maternal morbidity are hypertensive disorders, where preeclampsia/eclampsia are responsible for 77.2% of cases. The prevalence of obesity in pregnancy has ranges from 11 to 22%. According to reports, 35% of maternal deaths are obese mothers, having a 50% higher risk than people with normal weight. Preterm labor occurs in approximately 12.7% of all births, also presenting a risk to life. These disorders during pregnancy also have a serious impact on the health of newborns so that, exposures to various environmental factors (extrinsic and intrinsic) during early prenatal and postnatal stages are risk factors for the development of chronic diseases. Epigenetic factors (like DNA methylation and histone modifications) are etiological candidates for chronic diseases development through the regulation of fetal programming and developmental plasticity processes. DNA methylation is a heritable epigenetic mark used by the cells to control gene expression and it has an important participation in mammalian development.

Here, we showed part of a pilot study heading for the analysis of DNA methylome profiles from whole blood of children under three years old that faced adverse maternal conditions during pregnancy such as obesity, preeclampsia and preterm labor. For this, the assessing method used was the DNA modification with sodium bisulfite and methylation microarray chip analysis (Infinium Human Methylation 450K BeadChip of Illumina). Bioinformatics and statistical analyses were performed using GenomeStudio and Partek softwares.

The most important differentially methylated CpGs found are located on the following genes, for each group: 1. Obesity: DDX11, MAD1L1, NAT14 (hypermethylated) and FMN2, MAML2, TRIM10 (hipomethylated); 2. Preeclampsia: PSORS1C1, MYRIP LOC391322 (hypermethylated) and ZHX2, MIR654, MAML3 (hipomethylated); and 3. Preterm Labor: CLDN4, SGK3, OCA2 (hypermethylated) and MLLT1, TBC1D16, SERPINA10 (hipomethylated). Our findings suggest that obesity is the adverse maternal condition that has the most different methylation profile, compared with the control group. Thus, the consequences that this conditions may have on the child health are yet to be elucidated. The studies of DNA methylome at early childhood are strongly necessary to understand the etiological factors related to chronic diseases that develop in adulthood and that could be early programmed during prenatal exposure insults.

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# Gcn4-dependent transcriptional activation restores amino acid imbalance provoked by the lack of LEU3.

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In the yeast *Saccharomyces cerevisiae* Leu3 is a transcription factor that regulates genes involved in branched chain amino acid biosynthesis and ammonia assimilation. It has been proposed that the activity of Leu3 is positively regulated by α-IPM, the product of the first step in leucine biosynthesis. Current results from our laboratory have shown that in a *leu3*Δ *strain*, an amino acid imbalance is observed, inducing transcriptional activation of biosynthetic genes such as *HIS4*. In a double *leu3*Δ *gcn4*Δ mutant, *HIS4* expression is not induced, confirming that Leu3 deletion induces Gcn4-dependent transcriptional activation response (González et al 2016, in preparation).

In order to characterize the regulatory mechanisms displayed by Leu3, we identified the genes whose transcripts are up- or down-regulated in a *leu3*Δ mutant, in a strain that produces an excess of α-IPM, and in second strain that does not produce α-IPM, on different nitrogen sources during exponential phase.

Our results allowed the identification of the group of genes under Leu3-αIPM control. It was confirmed that the lack of Leu3 results in amino acid deprivation, when cells are grown on glutamine. Deprivation activates Gcn4-dependent expression of genes involved in amino acid biosynthesis, indicating that Leu3-dependent negative regulation is indirect and it acts through Gcn4-dependent positive control.

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# Functional analysis of the EhTRF-like III protein of Entamoeba histolytica

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Telomeric Repeat Binding Factors (TRF) bind to double stranded DNA sequences in the telomeres and play a critical role in telomere length regulation, chromosomes end protection, prevent chromosomes fusion, sensors of DNA damage, and regulate senescence or aging. In *Homo sapiens* two paralogues TRF1 and TRF2 bind to specific sequences in the telomeric region. Phylogenetic analysis have shown that *E. histolytica* contains three genes encoding proteins with high identity to TRF proteins dubbed EhTRF-like I, II, and III. In this work we selected and over-expressed the EhTRF-like III protein in *E. histolytica* trophozoites to gain insights into their cellular function in this parasite. Using Western Blot and immunofluorescence assays we observed that EhTRF-like III protein is localized in the nucleus mainly in the perinuclear region. EMSA assays demonstrated that this protein form specific DNA-protein complexes. Finally, we observed a lengthening of the stationary phase in the clones overexpressing the TRF-like III. These data show evidence that EhTRF-like III protein could be involved in the regulation of growth of *E. histolytica*.

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# The mir-143/145 cluster regulates the actomyosin cytoskeleton dynamics in prostate cancer cells

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MicroRNAs (miRNAs) are small non-coding RNAs which regulate the expression of mRNAs containing complementary sequences. Disruption of miRNA function is associated to many human diseases, including prostate cancer (PCa). PCa is considered to be a frequently diagnosed cancer in males with high mortality worldwide; however, the molecular mechanism responsible for prostate tumorigenesis and progression remains unclear. Increasing evidence has shown that miR-143/145 cluster play an important tumor suppressor role in PCa. In this work, we found that miR-143/145 overexpression on PC-3 cells decreases the viability and growth of spheroid cultures. Furthermore, miR-143/145 inhibited PC-3 cell proliferation but not induced apoptosis as measured by high-content screening. Cytoskeleton remodeling (CKR) is highly involved in processes related to carcinogenesis, as proliferation and migration. Because miR-143/145 cluster has several potential targets involved in CKR, we focused on phospho-myosin Light Chain2 (pMLC2) protein, a key downstream modulator of the actomyosin dynamics. Expression of pMLC2 was higher in PCa and RPE1 epithelial cells when transfected with miR-143/145 mimics. Moreover, in fibronectin micropattern assays pMLC2 localization changed in cells expressing miR-145. This change on the pMLC2 expression and localization due to miR-143/145 could be significant for tumor cell adhesion, directly affecting processes such as proliferation and migration. Thus, these results may explain the tumor suppressor role of miR143/145 cluster through regulation of the actomyosin cytoskeleton dynamics.

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# Identification of the AtPAO2 uORF peptide in Arabidopsis seedlings

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Putrescine, spermidine, and spermine are the most abundant polyamines (PAs) in nature. In plants, these amines participate in embryogenesis, growth and development, and in biotic and abiotic stress tolerance. Plant PAs are catabolized by cooper-dependent diamine oxidases (DAOs) and flavin-dependent polyamine oxidases (PAOs). The products derived from PAs catabolism are aminoaldehydes, diaminopropane, and hydrogen peroxide. *Arabidopsis thaliana* has five genes (*AtPAO1-AtPAO5*) encoding PAOs, which contribute to PAs homeostasis and participate through their catabolic products in physiological processes such as growth, signaling, and plant stress responses. Plant PAOs are regulated at multiple levels including transcription and translation. In particular, *AtPAO2* is translationally regulated by an upstream open reading frame (uORF) located in the 5´-UTR region. An uORF is a putative protein-coding region that repress the translation of the downstream ORF. The interest in studying the regulation mediated by plant uORFs is increasing, while there are no reports showing the detection of the uORF peptides *in planta*. In the present study, we show evidence of *in vivo* translation of the AtPAO2 uORF peptide. The AtPAO2 uORF peptide was immunodetected in flowers, silique, leaves, and immature buds of 45-day-old Arabidopsis plants. Interestingly, in 7-day-old whole seedlings we detected two bands corresponding to a doublet and a tetramer of uORF peptide. Thus, Western blot data suggest that the peptide is able to oligomerize *in planta*. The self-interaction of the peptide-encoded by the AtPAO2 uORF was confirmed by yeast-two hybrid analysis. These findings will contribute to our understanding on the specific mode of action of the AtPAO2 uORF peptide in the translational regulation.

# Inhibition of primary myoblast differentiation by *Trichinella spiralis* muscle larvae excretory-secretory products

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*Trichinella spiralis* is an intracellular parasite of mammalian skeletal muscles. Infection by L1 larvae eventually transforms the infected cell into a structure called nurse cell which allows parasite survival for long periods of time by inducing structural remodeling and gene expression changes in the infected muscle. In this process, the participation of excretory-secretory products (ESP) released by muscle larvae stage (ML) has been suggested. To elucidate the cellular processes altered by this parasite, we developed primary mouse myoblast cultures as a model to evaluate the changes at genetic expression level induced by ML ESP during differentiation from myoblast to myotubes.
The hind limb muscles were collected from 2-4 day old BALB/c mice to establish primary myoblast cultures. Cultures were treated with ML ESP at days 0 and 4 of differentiation. Total RNA and protein were isolated from ESP-treated and -untreated primary myoblast cultures after 8 days under differentiation conditions. A cDNA microarray assay was performed to evaluate gene expression changes. Data analysis were carried out by using different bioinformatics tools and some of the changes observed were validated by qRT-PCR and western blotting (WB).
ESP treatment produced a 50% reduction in myotube formation. In addition, shorter and thinner myotubes with fewer nuclei were observed. Altered expression was found in genes associated with different cellular processes like actin cytoskeleton regulation, cell cycle, apoptosis, MAPK signaling pathway, JAK/STAT signaling pathway and cell development. Expression changes in myogenic regulatory factors (Pax7, Myf5, MyoD, and Miogenin) and cytoskeleton proteins (Actin and Myosin) were also observed and validated by qRT-PCR and WB.
These results show some of the changes in primary myoblast cultures induced by ESP, which may occur in the nurse cell development.

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# Transcriptional regulation under normal and modified wort fermentation of two yeast strains used in beer industry

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Nowadays beer industry move around billions of dollars in the global market (Brewers Association. Boulders. CO. Estimate around 105.9 billion only in USA during 2015). For this industry the development of new products, with different flavors and composition, and the cheapening of production are only some of the new goals. Analysis of the process and characterization of the yeast strains used during fermentation are essential to competitive production.

In lager beer production, several strains of *Saccharomyes pastorianus* are used. These strains are the result of a natural hybridization between *Saccharomyces cerevisiae* and another member of the *Saccharomyces* family (*Saccharomyces eubayanus*, putatively). Even if these strains do have a common origin, previous research shows that different strains of *S. pastorianus* have different transcriptional regulation which results in products with organoleptic variants.

By RNA sequencing, using Next-Generation Sequencing (NGS) technology, we are going to analyze the transcriptional regulation of two *S. pastorianus* strains that are used in the brewing industry under normal and modified wort fermentation. Analyzing the transcriptome of these yeasts under the above mentioned conditions will allow us to identify critical regulation pathways that could be used to find new methods of production that could improve the time of fermentation and/or the final product.

**Keywords:** *S. pastorianus*; transcriptional regulation; organoleptic variants; wort fermentation.

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# Functional characterization of *Candida tropicalis MNN4*, *OCH1 and PMR1*

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*Candida albicans* and *C. tropicalis* are yeast-like organisms that can cause severe infections in immunocompromised patients. The cell wall structure and composition are critical factors for the interaction with host tissues and components of the immune system. In *C. albicans*, the cell wall has an inner layer of chitin and β-glucans, and an outer coat composed of mannoproteins, i.e., proteins that are mannosylated by the glycosylation pathways. *C. albicans OCH1* is involved in the synthesis of the *N*-linked mannan outer chain, and is required for cell wall integrity and virulence. *MNN4* is involved in the regulation of the phosphomannosylation pathway that adds phosphomannan moieties to both *N*- and *O*-linked mannans. This cell wall component is required for proper phagocytosis by macrophages. On the other hand, *PMR1* encodes a P-type $Ca^{2+}$/$Mn^{2+}$-ATPase and is required for glycosylation and virulence.

The *C. tropicalis* genome contains three putative genes with significant similarity to *C. albicans OCH1*, *MNN4* and *OCH1*. Thus far, there is not information about the cell wall assembly and mannosylation pathways in *C. tropicalis*; therefore, the study of the putative orthologs to *OCH1*, *MNN4* and *PMR1* is relevant to understand how the mannosylation and phosphomannosylation pathways contribute to *C. tropicalis* virulence and immune sensing.

In the present study, we conducted the complementation of *C. albicans mnn4*Δ, *Caoch1*Δ, and *Capmr1*Δ with *C. tropicalis MNN4, CtOCH1* and *CtPMR1,* respectively, under the control of *C. albicans ACT1* promoter. Our results suggested that the wild-type phenotype was restored in all cases.

We also performed the Ct*MNN4* disruption, and our results indicate the mutant cells lost the ability to bind Alcian blue, phosphomannan content is affected and phagocytosis by human macrophages and cell line Raw 264.7 is diminished. This result confirms that *CtMNN4* is a regulator of phosphomannosylation in *C. tropicalis.* We also disrupted *CtOCH1* and our results indicate these mutant cells have an aberrant cell wall structure, with decreased mannan content and higher levels of β-glucans, decreased ability to bind alcian blue and a temperature sensitive phenotype, being unviable at 42 °C. Finally, we disrupted *CtPMR1* and this mutant has 9-fold increase in chitin content and decreased mannan content, null ability to bind the Alcian blue dye, a temperature sensitive phenotype, and forms cellular aggregation in YPD medium. This results suggest that the *N*- and *O*- linked glycosylation are critical for stability of *C. tropicalis* cell wall. Our results also suggest that phosphomannans are present in both *N* and *O*- linked glycans and are critical for macrophages recognition, even more than in *C. albicans*.

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# Functional analysis of the silencing protein Abf1 in the fungal pathogen *Candida glabrata*.

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*Candida glabrata* is an opportunistic fungal pathogen that has become increasingly frequent in the last three decades in immunocompromised patients. *C. glabrata* is able to adhere to host epithelial cells *in vitro*, which is an important factor for its virulence. This ability is mediated primarily by Epa1, a cell wall protein which is part of a large family of adhesins. Our laboratory strain contains at least 23 *EPA* genes that encode putative adhesins. Most of these *EPA* genes are localized in subtelomeric regions, and are subject to subtelomeric silencing.

In *C. glabrata* the subtelomeric silencing is mediated by the SIR complex (Sir2, Sir3 and Sir4), Rap1 and Rif1. In addition, in our lab we characterized *cis*- acting elements that are independent of subtelomeric silencing. The protosilencer Sil2126 found in the right telomere of chromosome E ($E\text{-}_R$), between *EPA3* and the telomere, which depends on the yKu70 and yKu80 proteins and this particular subtelomeric context. Another *cis*-acting element is found in the intergenic region of *EPA1* and *EPA2* in the chromosome $E\text{-}_R$, called negative element (NE). This element also depends on yKu70 and 80 proteins. We found several putative binding sites for Abf1 (ARS binding factor 1) in Sil2126 and in several other regions throughout the cluster formed by *EPA1, EPA2* and *EPA3*. In *Saccharomyces cerevisiae*, a yeast phylogenetically closely related to *C. glabrata*, Abf1 is a multifunctional, site-specific DNA binding protein essential for cell viability which plays a role in transcriptional activation, DNA replication and gene silencing. These activities are conferred by the C terminus end of the protein.

We are interested in determining if *ABF1* is required for Sil2126 silencing activity, and whether it is essential for cell viability in *C. glabrata*. To do this, we established a plasmid loss assay. We have also constructed mutants in the C-terminus of Abf1 to determine whether this domain is important for subtelomeric silencing and for Sil2126 silencing activity.

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# The role of SERCA pumps in the resveratrol-mediated antitumoral effect in breast cancer cells.

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$Ca^{2+}$ is a highly versatile intracellular signal that operates over a wide temporal range to regulate many different cellular processes such as differentiation, growth and cell death. The involvement of $Ca^{2+}$ in so many fundamental cell processes naturally demands its efficient and precise control. The $Ca^{2+}$-ATPases from the sarco/endoplasmic reticulum (SERCA) are fundamental for maintaining intracellular Ca2+ homeostasis by pumping Ca2+ into the ER. It has been reported alterations in the expression of SERCA pumps in different types of cancer: oral, lung, colon, stomach, central nervous system, thyroid, breast, and prostate.

Resveratrol (RSV) is a phytoalexin produced by a wide variety of plants in response to stress situations. It can modulate multiple cellular processes, including apoptosis, cell cycle progression, inflammation, and angiogenesis. It affects the processes underlying all three stages of carcinogenesis; namely, tumor initiation, promotion and progression.

In this work, we used the breast cancer cell lines MCF-7 and MDA-MB-231 to evaluate whether RSV treatment affected the mRNA expression levels of two specific isoforms of SERCA (SERCA2b, an ubiqitous isoform and SERCA3, a tissue specific isoform). Results demostrate that RSV treatment increases the expression of the tissue specific isoform in both cell lines, but not the expression of ubiqitous isoform in a time and concentration dependent manner. Moreover, RSV treatment decreases cell viability, it triggers apoptosis and changes in cytosolic $Ca^{2+}$ levels, as well as changes in the capacity for $Ca^{2+}$ release by the ER were detected.

This data show, for the first time, an intracellular calcium management-mediated protective effect of resveratrol in breast cancer. Nevertheless, deeper research is needed to understand the molecular mechanisms underlying this effect.

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# Transcriptional factor TFIIB1 is involved in the response to osmotic stress in *Arabidopsis thaliana*"

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Plants as sessile organisms have strategies to maintain homeostasis during its life and respond to adverse external stimuli such as biotic and abiotic stresses. Abiotic stress conditions such as a drought, extreme temperatures and salinity have adverse effects on the growth and production of land plants. Transcription factors (TFs) play central roles in gene expression by regulating downstream gene expression via specific binding to *cis*-acting elements in the promoter of target genes. Furthermore, the transcription factor activities are coordinately regulated at various steps to fine-tune signal transduction pathways in diverse cellular signalling networks for optimal growth and survival under negative specific environmental conditions. TFs have the function of acting as regulators of the expression of various effector molecules involved in the perception and signal transduction of stress. In our laboratory, we isolated 15 T-DNA mutants from *A. thaliana* that shown a phenotype of insensitivity to the disaccharide trehalose *(tin= trehalose insensitive)*; in one of the mutants *(tin14),* the T-DNA is inserted close to the gene that coding for the basal transcription factor *TFIIB1*. Through *in silico* analysis in the genome of *A. thaliana,* we found that there are 14 TFIIB-like proteins that have been phylogenetically categorized into the TFIIB, Brf, and Rrn7/TAF1B/MEE12 subfamilies. By RT-PCR experiments on wild plants from *A. thaliana*, we have determined that the *TFIIB1* gene, is induced by dehydration and salinity (NaCl) stress conditions and also an alternative version (generated by alternative splicing) of the original transcript, is synthesized by retention of third intron. We propose that alternative TFIIB1 form, acts as a negative regulator of gene expression from "housekeeping" genes to lead the expression of genes involved in tolerance to abiotic stress. Using microarrays technology, we compared the transcriptome of the mutant and wild type plants, both normal and under stress conditions. Furthermore we evaluated germination, dry and fresh weight, lipid peroxidation (MDA) and measuring maximum efficiency of PSII. The results obtained to date make us propose that the basal transcription factor TFIIB1 is an early response factor in the signaling cascade during abiotic stress.

# Role of Gln3, Gat1 and Ure2 in the regulation of nitrogen metabolism in *Lachancea kluyveri*

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Yeasts grow differently according to the quality of the nitrogen source available in the media, absorbing first rich nitrogen sources over poor ones. A rich nitrogen source is defined as the one that is well transported into the cell and requires fewer steps to reach glutamine or glutamate. Conversely, a poor nitrogen source is the one that is not easily transported and requires more steps to form glutamine or glutamate. The ability to preferentially use rich nitrogen sources over poor ones is known as Nitrogen Catabolite Repression (NCR). In *S. cerevisiae*, NCR is regulated by the Ure2 protein and by four GATA family proteins: two activators (Gln3 and Gat1) and two repressors (Dal80 and Gzf3). Our interest is to study how different yeasts use diverse nitrogen sources and how this process is regulated. We found that *Lachancea kluyveri* has orthologous genes encoding for GATA transcription factors Gln3, Gat1 and for the Ure2 protein. We have generated a collection of single and double mutant strains in these genes in order to evaluate its role in nitrogen assimilation when cells were grown on glutamine, ammonia or proline as sole nitrogen sources. In order to elucidate if there is a NCR -like mechanism in *L. kluyveri* we measured, by qRT-PCR, the expression of some of the genes involved in the transport and catabolism of some of the nitrogen sources tested. Preliminary results show that both *LkGAP1* and *LkMEP2* are regulated by GATA transcription factors Gln3 and Gat1.

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# Analysis of small RNA biogenesis machinery expression in maize somatic embryogenesis induction.

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Maize somatic embryogenesis (SE) is induced from 15 days-old immature zygotic embryos under high auxin levels. The regeneration capacity is highly dependent on the genetic background and initial explant. Deep sequencing of small RNAs (sRNAs) during SE induction and maintenance revealed profound changes in specific microRNAs (miRNAs) and repeat-associated siRNAs (ra-siRNAs). Suggesting that components of sRNA biogenesis pathways might be operating in a different way during somatic embryogenesis.

To test the response of developmentally distinct maize embryos on SE induction, their morphology and cellular anatomy were analyzed by histological techniques. Different responses were observed among explants and only the 15 days-old immature zygotic embryo showed embryogenic callus formation. To evaluate the expression of sRNAs machinery components we focused mainly on Argonaute (AGO), Dicer-like (DCL) and RNA-dependent RNA polymerase (RDR) proteins. Based on a phylogenetic reconstruction and sequence identity between protein sequences, we found 5 RDR, 5 DCL and 24 AGO genes in maize with putative homology relationships to the *Arabidopsis* proteins. The transcript expression profile of some of these enzymes was analyzed by qRT-PCR during the induction and maintenance of maize embryogenic callus. Expression changes were observed to different extent for all transcripts upon SE induction. Suggesting readjustments in sRNA biogenesis pathway enzymes. Overall, our findings pinpoint a putative role of sRNAs biogenesis pathway, the developmental switch of appropriate tissues within the maize immature zygotic embryo towards a successful somatic embryogenesis induction

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# Molecular characterization of the *SIR* complex in *Candida glabrata* clinical isolates

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## Abstract

*Candida glabrata* is an opportunistic pathogen capable of producing superficial and disseminated infections in inmunosuppressed people. An important virulence factor for *C. glabrata* to establish the infection is the expression of adhesins, which mediate adherence to epithelial cells and plastic surfaces such as silicones and medical devices. *EPA1* gene encodes the Epa1 adhesin, which is responsible for 95% of the *in vitro* adherence to epithelial cells. *EPA1* belongs to a large family of related genes (*EPA* genes), most of which are localized close to telomeres,causing repression of its transcription due to subtelomeric silencing. In *C. glabrata* there are three Sir proteins (Sir2; Sir3; Sir4), which are essential for subtelomeric silencing.

We are interested in understanding the mechanisms of regulation of the *EPA* genes expression by the Sir proteins. We have analyzed the ability to adhere to epithelial cells in a collection ofclinical isolates, under conditions where our reference strain (BG2) does not express the *EPA1* gene. We found some isolates that express several subtelomeric *EPA* genes simultaneously and these isolates are hyperadherent to epithelial cells. We cloned the *SIR2*,*SIR3 and SIR4* genes of five hyperadherent isolates and control strains in replicative vectors for *C. glabrata* to determine if they can complement a null mutation in each of these genes in the BG2 background. We found that the *SIR2* and *SIR4* alleles of these clinical isolates are able to complement our *sir2*Δ and *sir4*Δ mutant respectively. However, we identified a *SIR3* allele in one hyperadherent clinical isolate which is unable to complement our *sir3*Δ mutant for repression or a reporter gene located in the subtelomeric region of the right arm chromosome E. We will discuss the implication of these findings.

# Basidiocarp development in *Ustilago maydis*: A transcriptional approach.

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*Ustilago maydis* is a Basidiomycota pathogenic fungus of corn and teozintle. The fungus has a nonpathogenic saprophytic life cycle, and other pathogenic infective stage which carries out within its host. This phase ends when germination of teliospores with formation of a phragmobasidium to give four meiotic product.
Although *U. maydis* was classified as a non-forming basidiocarp fungus, Cabrera-Ponce et al (2012), showed that when *U. maydis* was grown in dual cultures with an embryogenic maize callus under controlled growth conditions, it has the ability to form basidiocarps. The developed structures showed the presence of a hymenium composed of skeletal hyphae, generative hyphae, clamp connections, hyphal pore, and more impressive of holobasidias (not phragmobasidia), to give rise to basidiospores and thus complete its cycle.
One of the tools mostly used to identify the genes involved in the regulation of of development are microarray analyses. Using several online programs such as MIPs, FunCat. JGI, Smart; Pfam, NCBI, we analyzed the genetic changes occurring in the fungus when is transformed from the yeast stage to young basidiocarp and its its further establishment as a mature basidiocarp. 2002 were found to be regulated in the primary step (yeast to young basidiocarp) and 1064 differential genes were regulated in the last developmental stage (young basidiocarp to mature basidiocarp). Classification of the genes using FunCat software gave the following categories: Metabolism and energy, Cell cycle; Transport, Transcription, Proteins, Biogenesis, Signal transduction and interaction with the environment, Cell rescue, Differentiation and Unclassified proteins The mostly represented categories were Unclassified (31%), and Metabolism and energy with about 25%.
Search of *U. maydis* homologous genes to those previously described as important in the formation of fruit bodies in other fungal models, such as those encoding ortho-methytransferase and fatty acid synthase, differentially expressed in *Copriniopsis cinerea*; hydrophobin regulated in *Schizophillum comune,* gave positive results. These result suggest that formation of basidiocarps in *U. maydis*, possibly involves similar mechanisms as happens with members of the Ustilaginomycotina subphylum.

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# Patterns of gene activation in hybrid embryos of *Arabidopsis thaliana*

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After pollination, the fusion of the parental egg and sperm produces an embryo. During the first days of development, the embryo starts to be transcriptionally active in a process called Zygotic Genome Activation (ZGA). Functional and transcriptomic data have shown gradual activation of the paternal genome spanning days after fertilization. During this time, genes are differentially activated at distinct moments as the embryo matures.

In order for ZGA to be investigated at the genomic level, SNPs present in different Arabidopsis accession are used to differentiate parental contributions using hybrid crosses. In our lab, we compared isogenic and hybrid gene activation patterns and observed that hybridization can greatly influence the kinetics of paternal allele activation, i.e. different hybrids have different activation patterns. Although some hybrid combinations have a similar activation pattern for a specific gene, this pattern can be quite different in a different hybrid combination. These results conciliate contradictory conclusions from previous work on this topic.
So far, we have only sampled a small amount of the possible Arabidopsis intraspecific hybrid diversity. I'm currently working on determining the extent of variation of paternal allele activation patterns among a wider spectrum of hybrids. I have already constructed multiple gene reporter lines that I will use as pollen parents to determine the activation kinetics of the paternal genome. I am now crossing these lines to multiple *Arabidopsis thaliana* ecotypes selected based on their molecular differences. For the hybrids with the most divergent activation profiles, I will perform a comparative transcriptomic analysis of early and late activating hybrids.
This project is a first step towards the identification of the mechanisms responsible for differential gene activity in hybrid genomes, and their possible repercussions on hybrid vigor.

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# Does the accumulation of trehalose in *Ustilago maydis* change the virulence and the response to stress?

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*Ustilago maydis*, is a basidiomycete fungus that infects corn, is responsible of the corn smut or “huitlachoche” in Mexico. This fungus is a well-established model organism for the study of plant-microbe interaction, its genome was sequenced in 2006. The advantages of *U. maydis* compared to other phytopathogenic fungi are many, such as: it can grow in a defined media as haploid budding cells, produces symptoms in young plants in a few days, it shows high frequency of homologous recombination and recently huitlacoche has been considered a delicacy.

The non-reducing carbohydrate trehalose (α-D-glucopyranosyl-(1→1)-α-D-glucopyranoside) plays a dynamic cellular role, it is useful as a carbon storage and besides it works like a molecular chaperone, protecting the cellular integrity against several environmental stresses. It is not exactly known how the trehalose protects the cell, the most accepted theory talks about water replacement from biopolymers, besides the trehalose is widely distributed in plants, bacteria, archaea and fungi. The metabolism of trehalose in fungi starts with the enzymes Tps1/Tps2, which transform the glucose-6-phosphate and glucose-UDP in one trehalose molecule, once the sugar is not needed in the cell any more, the trehalose is degraded in two glucose molecules by the enzyme trehalases classified as neutral and acid, according to the range or pH where they are more active, and also related with the cell space where they are located.

The catabolism of trehalose in *U. maydis* is conducted by the trehalases Nth1 (Um11661.1) and Ath1 (Um2212), according to the *in silico* data. *Δnth1* mutants showed a higher concentration of intracellular trehalose, they are more virulent in corn and are more tolerant to oxidative, cell wall and osmotic stresses. In order to study the physiological effects in *U. maydis* mutants unable to hydrolyze trehalose, we constructed the mutant *Δath1* and by recombination *in planta*, the double mutant *Δnth1Δath1*. Now, we are conducting the experiments to study the phenotype of these mutants, regarding the response to stress, changes in cell morphology with emphasis in the virulence.

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# Expression profiles of tasiR-ARFs pathway-related genes during maize somatic embryogenesis

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Somatic embryiogenesis (SE) is a developmental process where somatic cells change their normal fate and undergo restructuring to generate embryogenic cells. These cells are able to undergo morphological and gene expression changes that result in the formation of somatic embryos and regeneration of new plants. The mechanisms underlying the signals for the location, differentiation and fate of pluripotent cells are poorly understood. Auxins play a vital role for plant growth, tissue differentiation and embryo patterning. Their physiological effects are mediated by transcription factors termed auxin response factors (ARF) that bind auxin responsive elements within auxin response gene promoters. The regulation of some ARFs involves trans-acting (ta)-siRNAs-mediated gene silecing. The ta-siRNAs originated from *TAS3* gene transcription, miRNA 390-guided cleavage of primary transcripts and targeting ARF3/4 are termed tasiR-ARFs. In maize, the tasiR-ARFs generated on the upper (adaxial) side of leaves move intercellularly to the lower (abaxial) side to create a gradient of ARF3 repression that patterns the leaf polarity

The aim of this study was to analyze whether there is differential expression of the tasiR-ARF pathway components in maize plant regeneration through SE. To this end, maize SE was induced from 15-18 day after pollination inmature zygotic embryos from Mexican landrace Tuxpeño, cultivar VS-535. The embryos were placed in medium supplemented with synthetic auxin 2,4D and kinetin in darkness to form embryogenic masses. After eight months of callus subculture, the callus was transfered to medium with gradual reduction of hormones and incubated under photoperiod (16 h light/8 h dark). The ES developmental stages were determined by histological analysis. Total RNA was extracted from each SE developmental stage and reverse transcribed to perform final point PCR expression analysis for eight TAS3 genes, five members of the maize ARF3 gene family, miR390 precursor transcripts and Leafbladeless (Lbl) which is required for tasiR-ARF biogenesis. Differential expression patterns *were observed for* precursor tasiR-ARFs and their targets *throughout* ES developmental stages, while Lbl expression was constant. These results provide insights for a possible role of tasiR-ARFs in maize somatic embryogenesis and plant regeneration.

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# Locus-specific *Rxrα* promoter methylation analysis and its expression in the offspring's umbilical cord from high-fat diet-induced obese rat

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**Introduction**. Retinoid X receptor alpha (Rxrα) plays important roles during mammalian development, cellular differentiation, and homeostasis events. Specifically, Rxrα regulation has been implicated in placenta and organogesis embryonic development. Genetic deregulations through epigenetic mechanisms (DNA methylation) at certain gestational stages may compromises the life of the product to good term. DNA methylation modifications at specific regions of the Rxrα promoter have been associated to maternal dietary imbalance during pregnancy and adiposity development in the offspring. Thus may have repercussions in gene and protein expression that could be related to metabolic disorders manifestation in the course of life. Rodent obesity models are useful tools to discern the epigenetic mechanisms that regulate gene expression in relation to unfavorable phenotypes associated to diseases. **Materials and methods**. Twelve female Wistar rats at weaning age were used to feed at least three months and during gestation with normal diet (Control: CO, n = 6) and high-fat hypercaloric diet (Model of obesity: MO, n =6). Twenty-four pulls of newborn puppies umbilical cords divided by sexual dimorphism were collected for nucleic acids and protein isolations (♀CO, n=6; ♀MO, n=6;♂CO: n=6; ♂MO, n=6). MS-HRM, RT-qPC Rand Western blot molecular methods were performed to DNA methylation, RNAm and protein expressions assays. Statistical analyses were obtained comparing the mean values of arbitrary absorbance units of each group and applying one-way ANOVA and t-test probes by Graphpad prism software. **Results**. Locus-specific Rxrα methylation profiling obtained by MS-HRM assays showed two distintct profiles of methylation between most of the study groups (both controls and ♀MO) and the ♂MO group. Similar results were obtained from RNAm analysis where also, both controls and ♀MO groups expressed similar Rxrα levels in contrast with the ♂MO group that, it showed a significant reduction in the Rxrα transcrites. Likewise, a significant decrease over Rxrα protein expression were observed in the ♂MO group. **Conclusions**. Males offspring exposed to a maternal high-fat diet-induced obese rat are more sensible than females offspring to Rxrα variations in its DNA methylation and transcrites and protein expressions. Therefore, it is suggested that males may be more prone to develop various diseases in adulthood due to the variations specifically in Rxrα.

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# Understanding *Candida glabrata* resistance to oxidative stress through *CTA1* gene regulation

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## Abstract

*Candida glabrata* is a haploid yeast found as a commensal in healthy individuals but causes serious infections in immunosuppressed patients. It is the second most common cause of candidiasis. *C. glabrata* has a well-defined oxidative stress response (OSR), which include enzymatic and non-enzymatic mechanisms. *C. glabrata* has one catalase, *CTA1*, which functions as a scavenger of $H_2O_2$ to maintain the redox balance in the cell. *CTA1* expression is induced in the presence of oxidative stress and by carbon source deprivation. *CTA1* has an upstream intergenic region of 4.5 kb. This intergenic region is much longer than the average of 450 bp intergenic regions in *C. glabrata*. The resistance to oxidative stress generated by $H_2O_2$ is mediated by *CTA1*, and is much higher than *S. cerevisiae* or *C. albicans*. In fact, in a heterologous complementation assay in *S. cerevisiae*, *CgCTA1* expressed in *S. cerevisiae* confers a higher resistance to $H_2O_2$. In order to understand how *CTA1* expression is regulated, we fused in a plasmid the *CTA1* intergenic region with GFP and generated 5'-3' and 3'-5' deletions. We then analyzed the activity of the *CTA1* promoter in the presence of $H_2O_2$ by FACS. We determined that the minimal promoter is located at -1 kb from the ATG. We identified 2 positive regulatory cis acting elements located between -4 kb and -3.3 kb and between -1.34 kb and -1 kb. There is negative regulatory cis acting elements between -3.3 kb and -3 kb. Identification of the transcriptions factors controlling the expression of *CTA1* will be discussed.

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# Stem loop: Molecular technique used for isolation and identification of small RNAs from *Giardia lamblia*

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*Giardia lamblia* is a flagellate protozoan that was adapted over time to a parasitic lifestyle. This organism causes the disease known as giardiasis in humans, but can also affect several vertebrate organisms. *G. lamblia* has been classified as a divergent microorganism and is considered a good model for gene regulation studies because it shares expression mechanisms identified in prokaryotic and eukaryotic organisms. As is the case of microRNAs, which are small RNA molecules that regulate genes expression and that have not been studied in detail in *G*. *lamblia*[1]. Some microRNAs (miR2, miR4, miR5, miR6, miR10) derived from small nucleolar RNA (snoRNA) were identified using programs predictors [1]. Stem loop is a molecular method used for identification and quantification of individual microRNAs in tissue or cultured cells[2]. In this work we use the technology for stem loop design, which allowed us to detect in a rapid and specific way the novel small nucleolar RNA (snoRNA) and its precursor the microRNA miR2 of *G*. *lamblia*. The small RNAs were isolated using two different methods (total RNA and microRNAs) and their identification was obtained by sequencing. Besides, it was determined by RT-qPCR that snoRNA GlsR17 is expressed constitutively during the 96 h of culture; while the mature microRNA (miR2) in trophozoites are only expressed in the first 48 h of culture. Given the importance of these small non-coding RNAs, their identification by stem loop as a simple tool, will allow to the future better understanding about of the features and functions, the stem loop-miRNA molecules will provide interesting subjects to study the gene regulation in *G*. *lamblia*, which are involved in the RNAi pathway and under any physiological condition.

[1]Saraiya *et al*., RNA. 2011. 17(12): 2152–2164. doi:10.1261/rna.028118.111
[2]Yang *et al*., 2014. PLoS ONE 9(12): e115293. doi:10.1371/journal.pone.0115293

# Characterization of KH-QUA2 domain of the U2 snRNP auxiliary factor 84 kDa (U2AF84) of *Entamoeba histolytica*

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The introns of *Entamoeba histolytica* possess conserved 5′ (GUUUGU) and 3′ (UAG) splice sites, but the branch point sequences (BS) lack such conservation. Therefore the polypyrimidine tract, SF1 (Splicing Factor 1) and the U2 snRNP auxiliary factors are essential for 3' splice site recognition. Recently, the snRNP protein U1A was tag-cloned and used in UV cross-linking immunoprecipitation (CLIP) assays coupled to tandem mass spectrometry analyses identified 32 amoeba splicing-specific factors including U2AF84, SF1, and U2AF33 (Valdés et al., 2014. J. Proteomics: 111, 30–45). We previously showed that U2AF84 silencing increased the splicing variants of some amoeba transcripts, suggesting that it is involved in intron retention via splicing inhibition. Unlike U2AF65 and Mud2, U2AF84 contains 226 extra amino acids of which 136 amino acids are very similar to the KH-QUA2 domain of the human SF1. We asked whether the KH-QUA2 domain of U2AF84 (84-KQ2) and the KH-QUA2 domain of SF1 (SF-KQ2) compete for BP binding, or interact with each other resulting in splicing increase or blockage. EMSA, UV/FH-CL and CLIP assays suggest that, in solution, 84-KQ2 prevent binding of SF-KQ2 to the BP, through an RNA-independent interaction; these KH-QUA2 protein-protein interactions were confirmed by far-WB. Furthermore, 84-KQ2 stabilizes the preformed interactions between SF-KQ2 and the BP blocking U2 snRNP/SF1 interchange. Finally, when wild type and U2AF84ΔC (without KH-QUA2 domain) were over expressed, we found respective accumulation or decrease of the pre-mRNA of some transcripts. We conclude that the 84-KQ2 domain is responsible for the splicing Inhibitory function that leads to intron retention.

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# Sweet taste perception genes: TAS1R2 and GLUT2 polymorphisms, carbohydrate intake and nutritional state in Mexican adults

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Abstract

The aim of the study was to assess the relationship between sweet taste perception gene variants, sugars consumption and nutritional state in Mexican adults. All the participants (n=206) recorded eating patterns and physical activity by completing three sequential 24-hour dietary recalls which were further reviewed in interviews where anthropometric, sociodemographic and clinical background were registered. We genotyped variants from genes involved in carbohydrate detection and transport, TAS1R2 (rs35874116) and GLUT2 (rs5400), respectively. Differential carbohydrate intake (g/day) was observed between gender ($p<0.001$). According to daily intake recommendations, carbohydrate caloric intake was insufficient (<55%) in Mexican adults and significantly different when compared to nutritional state ($p=0.040$). Comparisons between genotypes and nutritional state differed significantly only between TAS1R2 polymorphism and males ($p=0.044$) in which heterozygote individuals have a higher risk of obesity. In addition, a tendency for differential carbohydrate intake was observed for both polymorphisms regardless of gender and nutritional state. This is the first report showing the significant association between the TAS1R2 rs35874116 polymorphism and nutritional state in Mexican adults.

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# Analysis of phasin-like protein on Polyhydroxybutyrate production in *Azospirillum brasilense* Sp7

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**Introduction**. The Gram negative, α-proteobacteria *Azospirillum brasilense* Sp7 produces homopolymers of poly-β-hydroxybutyrate (PHB) up to 75% of the cell dry weight. PHB is intracellularly synthesized by three enzymatic reactions carried out by β-ketothiolase, acetoacyl-CoA reductase and PHB synthase. PHB polymer is stored as cytoplasmic granules and is covered by granule associated proteins (GAP): synthases, depolymerases, regulators and phasins. Phasins are the major proteins that coat PHB granule and stabilize it, probably generating an interphase between the cytoplasm and the hydrophobic core of the granules, preventing them from coalescence. Actually, several functions have been assigned to phasins, so, the aims of this work were analyze the function of a Phasin-like protein on the PHB production in *A. brasilense*.

**Methods**. Bioinformatics analysis were performed. NCBI Conserved domains, SMART and Pfam databases were used to looking for Phasin domains in genomic sequence of *A. brasilense*. Upstream and downstream flanking regions of AMK58_17045 gene were amplified in two parts, amplicons A (1499 bp) and B (1329 bp) by using the primers AMK58_17045FA/AMK58_17045RA and AMK58_17045FB/AMK58_17045RB respectively. Restriction sites *Pst*I/*Xma*I and *Xma*I/*Eco*RI were designed, respectively, to insert the amplicons A y B into suicide vector pSUP202, generating pSUPAB. This plasmid was movilized into *A. brasilense* Sp7 using *E.coli* S17-1. Deleting mutants were selected as Mishra *et al*. 2011. PHB content was measured as Law and Slepecky, 1961.

**Results and conclusion**. A total of six genes were found containing a Phasin domain (AMK58_17045, AMK58_04265, AMK58_04260, AMK58_07505, AMK58_13820 and AMK58_20940). So, was decided to delete the AMK58_17045 gene. PHB content was determined and showed an altered production in *A. brasilense* deleted mutant respect WT strain.

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# Gene regulation in *Ustilago maydis* by a MAPK pathway involved in pathogenesis, mating and morphogenesis

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Mitogen-activated protein kinase (MAPK) pathways are evolutionarily conserved, and one of the most important mechanisms of cellular signaling existing in eukaryotic organisms. The signal transduction processes where MAP kinases are involved, initiates with the sensing of environmental stimuli by receptors anchored to the cell membrane, and bound to heterotrimeric G proteins, they can interact with adaptor proteins, or directly activate a MAPKKK (MAP kinase kinase kinase); which in turn activates a MAPKK (MAP kinase kinase) by phosphorylation; and subsequently this latter protein phosphorylate MAPK (MAP kinase). Finally this kinase give rise to activation of transcription factors that induce or repress genes involved in the cellular adaptation or response to the sensed stimuli.

In fungi, the MAPK pathways are involved in different physiological and developmental processes, including: cell cycle, mating; cell wall integrity, sporulation, autophagy, morphogenesis, pathogenesis, and response to different forms of stress. In the present work we proceeded to identify all the genes regulated by the signal transduction pathway MAPK involved in mating, pathogenesis and morphogenesis (PMM) of *Ustilago maydis*. Our previous results demonstrated that mutants deleted in the different genes involved in this pathway are avirulent, and monomorphic growing constitutively as yeasts [1,2].

With the objective to identify the genes regulated by this pathway that had a role in dimorphism or/and pathogenesis, we made a comparison between the transcriptomes of a wild type strain and a *ΔUbc2* mutant affected in the interacting protein of this pathway by use of microarrays. By this methodology we identified 939 genes regulated directly or indirectly by this MAPK pathway. Of them, 432 were positively, and 507 were negatively regulated. By functional grouping, genes encoding cyclin dependent kinases, transcription factors, proteins involved in signal transduction, in the synthesis of cell wall and cell membrane, and involved in dimorphism, were identified as differentially-regulated.

Focusing on the dimorphism and virulence of the fungus, 60 of the 154 genes previously identified to be differentially expressed during the *U. maydis* dimorphism [3], were regulated by this MAPK; and 169 of the 933 genes described as clusters of pathogenesis, transcription factors, effectors, degradative enzymes, and secretory proteins (all them important for *U. maydis* pathogenesis and virulence in maize, and identified by different methodologies), were regulated by the PMM MAPK pathway.

These data reveal the importance of the PMM MAPK pathway in the morphogenesis and pathogenesis of *U. maydis*, and identify the genes specifically regulated in these processes.

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[2] Martínez-Espinoza AD, Ruiz-Herrera J, León-Ramírez CG & Gold SE (2004). MAP kinase and cAMP signaling pathways modulate the pH-induced yeast-to-mycelium dimorphic transition in the corn smut fungus *Ustilago maydis*. *Curr Microbiol* 49: 274-281.

**[3] Martínez-Soto D & Ruiz-Herrera J (2013). Transcriptomic analysis of the dimorphic transition of *Ustilago maydis* induced *in vitro* by a change in pH. *Fungal Genet Biol.* 58-59:116-125.**

# Identification of low molecular weight excretory-secretory products from *Trichinella spiralis* muscle larvae potentially involved in the inhibition of myotube formation in primary myoblast cultures

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*Trichinella spiralis* L1 larvae infect muscle cells and induce a series of changes which eventually lead to the development of the nurse cell. It has been suggested that muscle larvae (ML) excretory-secretory products (ESP) may be involved in the formation of this niche. However, the molecules responsible for these changes have not been identified.
With this objective ML ESP were separated into low and high molecular weight fractions using 30 kDa as a threshold and their effect in primary myoblast cell cultures was analyzed. Low molecular weight ML ESP were analyzed by mass spectrometry; their genes were cloned, and the expressed proteins were purified.
Both ML ESP fractions inhibited myotube formation in primary myoblast cultures. However, the low molecular weight ML ESP fraction prevented the elongation of the fused muscle cells while the high molecular weight fraction reduced the fusion of cells. Three proteins were identified by mass spectrometry from some of the low molecular weight protein bands chosen to be analyzed. Two of the identified proteins corresponded to cystatin-like proteins, whose genes belong to a small family of interrelated genes which encode proteins whose function have not yet been characterized. The corresponding genes were cloned, overexpressed and the proteins were purified to start their biochemical characterization. Overall, these results indicate that the low molecular weight ML ESP fraction induce changes during the *in vitro* differentiation of myoblasts and suggest that these proteins may also be involved in nurse cell formation.

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# Physiological roles for MXL-3/MAX as lipogenic factor that modulate organismal fat accumulation

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Hyperglycemia is a key risk factor to develop diabetes mellitus (DM) and metabolic syndrome. These diseases are result from an imbalance between the amount of energy ingested and consumed, a common feature in lipid dysregulation that affects energy homeostasis. Although glucose is the main fuel source in eukaryotes that upon oxidation provides energy, excessive glucose availability have detrimental effects (glucolipotoxicity) on organismal metabolism. In mammals, the transcriptional factor MAX, Myc-associated factor X, has shown that high glucose increase both abundance and association with its obligate heterodimer partner c-Myc to activate several glucose and *de novo* fatty acids genes. *Caenorhabditis elegans*, has two Max orthologs (MXL-3 and MXL-1) with high homology to mammals MAX. Interestingly, only MXL-3 is regulated by nutrient availability; however, its physiological role on lipid metabolism is not well understood. Here we shown that in response to high glucose, MXL-3 promotes lipid metabolism whereas metformin, considered an initial drug of choice for DM treatment, blocks its activity and improve lipid accumulation. In fact, we found that MXL-3 promotes lipid synthesis by activating SBP-1, the sterol regulatory element-binding protein (SREBP) in *C. elegans*, the major master regulator of lipid biosynthesis, and its targets lipogenic enzymes *pod-2* and *fasn-1*. Animals fed with high glucose showed augmented MXL-3 expression and nuclear localization that correlates with stored lipids levels, and mutations either in together with MXL-3 or SBP-1 abrogate the lipid storage defects associated with deregulated *mxl-3* expression. Together our results reveal a new physiological role for MXL-3 as lipogenic factor that modulate organismal lipid accumulation via SBP-1 in *C. elegans* and suggest that MXL-3, is involved in the generation of metabolic diseases, becoming an attractive therapeutic target to combat metabolic diseases such as obesity. DGAPA-PAPIIT:RN208214. CONACyT:CB-221953.

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# Germ-line BRCA 1 and 2 mutations in breast and ovarian cancer patients

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BRCA 1 and 2 deleterious mutations have been identified as risk factor for developing breast and ovarian cancer, the first and fifth cause of death in women worldwide, respectively. In México, breast and ovarian cancer constitute an important public health problem due to the 7700 death that they cause. According to previous reports, up to 10% of patients diagnosed with breast and ovarian cancer harbor a germinal mutation in BRCA1 or 2. Early detection of BRCA mutations allows for decision making towards taking primary prevention measures, such as mastectomy and oforectomy. The aim in this work was to analyze deleterious mutations in BRCA genes in patients that have been diagnosed with breast or ovarian cancer.

We analyzed 250 diagnosed patients with breast cancer and 50 with ovarian cancer by means of massive parallel sequencing, employing the Ion Torrent PGM instrument to analyze the full open reading frame of both genes. Twenty-one out of 250 (8.4%) breast and 4 out of 50 (8%) ovarian cancer patients bore a pathogenic mutation in the BRCA ORF. We were able to describe 17 deleterious mutations, 10 in BRCA1 and 7 in BRCA2. The most frequent mutation, c.66_67delAG, was found in four breast cancer patients. All the mutations that we sequenced had been previously reported and annoted in several databases. This is the first study describing BRCA1 and 2 mutations using massive parallel sequencing in a large cohort of mexican breast and ovarian cancer patients.

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# Functional interactions of NFAT, NF-kB and Sp1 proteins regulate the expression of IL-10 in U937 monocytes

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The interleukin-10 (IL-10) is a powerful anti-inflammatory key cytokine for the maintaining of cellular homeostasis. The use of bioinformatics programs has determined the presence of a great number of union elements to transcription factors (TF) in the IL-10 promoter, eg NF-kB, NFAT, CRE, GRE, AP-1, C/*EBP, Sp1 and* STAT3, among others; whose functions in the regulation of the IL-10 expression is not well understood. Previous studies in our research group showed that different stimuli such as LPS/$PGE_2$, cAMP and GM-CSF induce the expression of IL-10 in macrophages. *In vitro* studies of DNA-protein interaction demonstrated the presence of complexes in NFAT, AP-1, NF-κB, Sp1 y GRE sites. Also showed that c-Jun protein is present in the proximal composite NFAT/AP1 (-181/-161bp), and the presence of the p50 and p65 proteins in the NF-κB distal site (-1973/-1964bp); indicating that this stimulus can activate different transcription factors that bind to its site in the IL-10 promoter.

In this work we are studying the functional interaction of the proteins NFAT, AP-1, NF-kB, Sp1 and STAT3 in the IL-10 promoter region of -827/+38bp and -2049/-1937bp in U937 monocytes stimulated with LPS/$PGE_2$ through the use of the Chromatin Immunoprecipitation (ChIP) assay. We are also looking at whether these transcription factors can form multiprotein complexes and their relationship with IL-10 mRNA and protein expression; in order to understand the molecular mechanisms implicated in the regulation of this gene. The results showed that the IL-10 expression at mRNA and protein levels had an increase at 0.5h and the maximal peak of expression at 8h. Functional interactions were observed with the sites: NF-kB (-464bp), Sp1 (-636bp) and composite NFAT/AP-1 (-656bp), and we did not find functional interactions of transcription factors with sites: NFAT/AP-1 (-181bp) and NF-kB (-1973bp or -2049bp), nor with the STAT3 site (-88 or -272bp).

Immunoprecipitation (IP) experiments with anti-p50 helped identify the interaction with NFAT, Sp1 and p65; these complexes could be involved in the expression of IL-10. This evidence contributes to a better comprehension of the activation mechanism of the transcription of this interesting anti-inflammatory cytokine, and opens a wide field of study. In this regard, we will continue with the evaluation of the formation of multiprotein complexes, as well as the functional interactions with other sites in the promoter of IL-10.

**KEY WORDS**: ChIP, IP, IL-10, transcription factor, monocytes.

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# Postranscriptional regulation of Cyclin D1 protein in cells transfected with E6 oncogene from HPV type 6, 16, 18, and 52.

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Abstract

The eIF4E protein is a member of the eIF4F complex, whose activity is associated with the postranscriptional regulation of some gene implicatedwith the cell cycle transition, such as Cyclin D1.It has proposed that cervical cells infected with high-risk papillomavirus, the eiF4E transcription is upregulated through the increase in the c-myc protein levels as a result of the p53 degradation (Wang *et al*, 2013). Thereby, increased expression of eIF4E could affect the transport of specific transcripts linkedwith the cervical carcinogenesis, such as Cyclin D1, and Ornithine Decarboxilase (ODC). However, despite that eIF4E overexpression is a finding commonly observed during cell transformation, its activity is mainly associatedwith the phosphorylation at the serine-209, suggesting that the mechanism involved in the control of its activity during cervical cancer progression is not well known.

In this study, we proposed that E6 oncogene from high-risk papillomavirus modifies c-myc and ODC protein levelsby increasing theeiF4E expression, but also as a consequence of the increment in thephosphorylation levelat serine-209. The study model consisted in the transfection of two cell lines (MCF7 and HEK293)with E6 oncogene from low- and high-risk papillomavirus (6, 16, 18 and 52).Cell transfection was performed with cationic lipid, andtranscript and protein quantitative expression was evaluated by RT-qPCR and western blot, respectively.

The expression assayfrom the MCF7 and HEK293 models showed that even in the presence of E6 from high-risk papilloma, there was no significantly change in the level of the eiF4E transcript.Among the analyzed genes whose transcription is regulated by eiF4E, only the ODC1 expression showed a significantly increment in MCF7 cell line. At Protein level, although p53 protein degradation was confirmed in cell lines transfected with E6 from high-risk types, the reduction was not associated with the increment of eiF4E protein. However, the increased protein level of eiF4E correlated with its phosphorylation at serine 209 and with the augment of Cyclin D1 protein observed in clones withhigh-risk E6. These results suggest that E6 from high-risk types could modify the activity of a signaling pathway implicated in the control of eiF4E phosphorylation.

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# Molecular classification of histone metyltransferases

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The nucleosome represents the first level of chromatin organization in eukaryotes. Core nucleosome is composed of two copies of each H2A, H2B, H3 and H4 histones. Functional duality of the nucleosome are structural and regulatory and depends on diverse post-translational modifications (PTM) in the histone tails. These modifications include acetylation, phosphorylation, ubiquitinylation, sumolyation, methylation, among others. Histone methylation is executed by histone methyltransferases (HMT), which transfer methyl groups from S-adenosyl-L-methionine (AdoMet) to a lysine and arginine residues mainly of H3 and H4 histones. Thus HMT can be divided into two types: lysine (HKMT) and arginine (PRMT) methyltransferase histone. In addition a lysine residue may become mono, di or tri-methylated status and this is related with activation or repression transcription.

Some attempts to classify the HMT have the disadvantage that these employed a limited number sequences and organisms and do not show the molecular basis of this. The goal of this study is to establish a molecular classification of HMTs based on their domains and motifs of amino acid sequences of eukaryotic HMT.

By means of PSI-BLAST strategy, we analyzed 360 well characterized HMT from representative organisms of the most eukaryotic *phylum*. These were analyzed and classified in order to determine groups. Three main groups were formed separating: PRMT, HKMT and DOT1. i) PRMT family consists of seven members PRMT1 to PRMT7, ii) HKMT superfamily which include seven families SET1, SET2, SET7, SETD8, SUV39, SUV40 and EZ, iii) The DOT1 family.

Our results reveal that the classification of the histone methyltransferases falls into three distinct groups named DOT1, HKMT, and PRMT. This division is based of specific motifs of HMT proteins and suggests the diversification of their catalytic mechanisms. This study will aid to explore the differences in the catalytic mechanisms and protein interactions of HMTs, as well as help us to detect essential and non-essential regions of each group of HMTs established.

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# Phenotypic and transcriptomic analysis of the *atx1-1*$^{setm}$ mutant affected in lateral root development.

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Chromatin remodeling is important for plant growth and development, particularly for organogenesis (Wagner, 2003).Together with other histone modifications, the methylation of Lys residues acts as an important component of epigenetic changes to induce transcriptional repression or activation (Richards, 2002).The *ARABIDOPSIS HOMOLOG OF TRITHORAX (ATX1)* gene encodes histone H3K4 methyltransferase that is involved in the regulation of timing of root developmental processes, stem cell niche maintenance, and cell patterning during primary and lateral root development (Napsucialy-Mendivil, *et al*. 2014). Here, we present the phenotypic and transcriptomic analysis of the mutant *atx1-1*$^{setm}$, in which an inactive form of the ATX1 protein is expressed on *atx1-1* loss-of-function background. The primary root length in *atx1-1*$^{setm}$ mutant was 65% less than the wt and 40% less than the *atx1-1* mutant at 10 days old. In addition to the primary root growth phenotype stronger abnormalities in lateral root primordium development were recorded in *atx1-1*$^{setm}$ compared with the *atx1-1* mutant. We performed high-throughput sequencing (RNA-Seq) of the *atx1-1*$^{setm}$ mutant and the wt. The mRNA was extracted from roots of 14 day old plants from two independent experiments. We found 194 differentially expressed genes, among them 111 downregulated and 83 upregulated genes, with a fold change of 4 and *P* value of -0.005. We found a number of very interesting genes affected in the mutant, which are involved in root development: AGL (AGAMOUS LIKE) containing MADS-box, TCP (TEOSINTE BRANCHED, CYCLOIDEA, PROLIFERATING CELL NUCLEAR ANTIGEN FACTOR) and NAC (NAM ('no apical meristem'), ATAF1/2, CUC2 ('cup-shaped cotyledons 2') ) transcription factors. Finally, we found genes specifically expressed in pericycle that changed their expression in the background of the *atx1-1*$^{setm}$ mutant, among them 8 downregulated and 4 upregulated. The downregulated genes in the mutant are probably directly regulated by ATX1.

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# The *TIME FOR COFFEE* gene participates in the hydrotropic response regulation in *Arabidopsis thaliana*

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Plants are constantly exposed to environmental changes like light intensity, gravity, obstacles and low water content in the soil and they have developed a morphological plasticity to adapt to those changes. One of the ways in which plants contend with the fluctuating environment is by developing tropisms. Plant roots show positive hydrotropism in response to moisture gradients, a feature that is important in controlling their growth orientation for obtaining water. The hydrotropic response has an important role in establishing the structure of the root system, and thus, has implications on the ability of plants to survive under limiting water conditions such as drought stress. In addition, it has been reported that ABA, a water stress hormone, is a modulator of the mechanisms that integrate the hydrotropic response. To better understand how Arabidopsis responds to moisture gradients, we identified an altered hydrotropic response (*ahr2*) mutant of Arabidopsis in the screening system with a water potential gradient generated with glycerol (NM→WSM; NM, normal medium and WSM, water stress medium). The *ahr2* mutant showed higher sensitivity to exogenous ABA on germination and seedling establishment compared to wild-type. Consistent with this result, the *ahr2* mutant grown under drought conditions showed improved tolerance compared to the wild-type. The semi-dominance of *ahr2* is likely a gain-of-function mutation. Mapping of the *ahr2* mutant was done by single sequence length polymorphism (SSLP) and the *AHR2* gene was localized in chromosome III at the locus of the *TIME FOR COFFEE* (*TIC*) gene, which is therefore responsible of the *ahr2* phenotype. The complementation testing determined that *ahr2* and *tic-2* mutations are alleles of the *TIC* gene. TIC was previously described as a nuclear regulator of the circadian clock and was known that the circadian clock and abscisic acid pathways are reciprocally coordinated. Since the circadian clock is considered as a key regulator in different functional events and adaptation to various geographic environments, we propose that *TIC* could be a regulator of the hydrotropic response in Arabidopsis.

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# HLH-30/TFEB together $NAD^+$ relieves the blocked autophagic flux due to high glucose diet

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Autophagy is a highly evolutionarily conserved self-digestion process, which is essential for maintaining cellular homeostasis in response to metabolic stress through transcription factor EB (TFEB or HLH-30 in *Caenorhabditis elegans*). TFEB, known as a master regulator of autophagy-lysosome pathway, play a fundamental role in cell homeostasis by activation of genes that share a common regulatory motif in their promoter regions, namely the CLEAR (Coordinated Lysosomal Expression and Regulation). Evidences have shown that autophagy promotes both cellular survival, in response to a variety of stress conditions, and cell death. Although both effects have been found in mammalian cells exposed to high glucose concentrations (HGC), the molecular mechanism underlying this autophagy regulation is not well understood. In the present study, we determined if HGC could affect the expression and the nuclear localization of HLH-30/TFEB, and thus the autophagic flux in *C. elegans*. Unexpectedly, our data showed that both gene expression and nuclear localization of HLH-30/TFEB as well as its targets autophagy-related genes (*lgg-1, lmp-1, unc-51* and *pgp-2*) were significantly increased in *C. elegans* fed with HGC. Interestingly, despite an increase autophagy-related genes expression, the autophagy flux was decreased as observed by augmented SQST-1::GFP protein aggregates and was restored to control levels by extracellular supply of $NAD^+$ precursor. In summary, our results suggest that HLH-30/TFEB along with extracellular $NAD^+$ restore autophagic flux and provides a new insight for exploring the mechanisms of autophagy and NAD+ homeostasis, and it is also valuable in the development of innovative strategies to combat metabolic disease. DGAPA-PAPIIT:RN208214. CONACyT:CB-221953.

# *Phaseolus vulgaris* calreticulin: unraveling its role in nodulation

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Legumes establish a symbiotic relationship with specific soil bacteria called rhizobia. This symbiosis initiate when the plant roots exude flavonoids into the rhizosphere, then these are recognized specifically by rhizobia triggering the synthesis and secretion of lipochitooligosaccharides, known as Nod factors (NF). NF induce physiological changes in the root hair cells such as an increase in the influx/efflux of $Ca^{2+}$, $Cl^{-}$ and $K^{+}$ ions, pH changes, and rearrangements of the actin cytoskeleton, among others. Thereafter, the formation of a novel organ, the nodule occurs (1, 2). Previously, twenty one phosphoproteins were identified by a phosphoproteomic analysis (unpublished results). The relative abundance of some of these phosphoproteins increased (>1.3 fold) in response to NF treatment. Among these, we identified calreticulin (CRT), which relative abundance increased twelve times in *Phaseolus vulgaris* roots, after specific NF treatment during 10, 30 and 60 min. CRT is a highly conserved molecular chaperone, and a $Ca^{2+}$ binding protein which resides mainly in the endoplasmic reticulum (ER) and is expressed in all eukaryotic organisms. Hitherto, CRT has been reported that in plants participates in reproductive processes, tissue regeneration, abiotic stresses, innate immune response and during pollen tube growth in *Petunia flowers* (3, 4). Herein, the participation of one of the members of the *P. vulgaris* CRT gene family, *Pv*CRT08, was functionally analyzed during nodulation. *Pv*CRT08 promoter activity was detected in nodule primordia and in the central region of *P. vulgaris* root nodules, using a promoter β-glucoronidase gene fusion. In non-inoculated roots, the activity was found in the meristematic regions. Moreover, down-regulation of *Pv*CRT08 mediated by RNAi in transgenic roots inoculated with *R. tropici* at 21 days show that there is a significant increase in nodule number and nitrogen-fixing activity as well as in the size of the nodule diameter and biomass. These findings indicate that *Pv*CRT08 has an important role while the development and functionality of the nodules in *P. vulgaris* roots.

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# A novel class of non-coding RNAs in *Entamoeba histolytica*

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Circular RNA molecules (circRNAs) are a new class of non-coding RNAs, which are expressed in different cell-types and organisms. circRNAs may arise from intergenic or coding regions, introns, and UTRs through mechanisms poorly understood. Most circRNAs have been described in multicellular eukaryotes, yeasts, and the protists *Plasmodium falciparum* and *Dictyostelium discoideum*, but not in clinically relevant protists such as *Entamoeba histolytica,* the etiological agent of amoebiasis. We identified circular RNAs of *E. histolytica* which differ from previously reported circRNAs in that they are conformed by **f**ull-**l**ength **i**ntronic **c**ircularized **RNAs** (flicRNAs). The intron circle of *RabX13* (flicRX13) was characterized at large. We found that flicRX13 is generated co-transcriptionally and its abundance inversely correlates with that of its parental pre-mRNA and that of the accompanying spliced variant. Actinomycin D incubation of Entamoeba cultures blocked RabX13 transcription but flicRX13 remained longer times than corresponding splicing variants. To understand flicRX13 biogenesis, we overexpressed a mutant version of U2AF84 (a constitutive splicing factor involved in early spliceosome assembly, known to be a splicing inhibitor) and observed that both spliced and flicRC13 levels increased, confirming that flicRNAs are splicing byproducts, and suggesting their possible post-splicing origin. To explore this possibility, we overexpressed a debranching-deficient Dbr1, otherwise implicated in the biogenesis of other circRNA species. Unexpectedly, debranching-deficient Dbr1 caused an increase in flicRX13 levels, probably due to the intronic GU-rich 5´ss which cause debranching escape in other circRNA species. Our results suggest that *E. histolytica* flicRNAs biogenesis involves the activity of the Dbr1 enzyme in a post-splicing event.

# Functional Analysis of Mir-122 on Radioresistance of the Breast Cancer Cells.

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Resistance to radiotherapy is one of the major challenges in breast cancer control. MicroRNAs (miRNAs) are master regulators of gene expression at the translational levels. Some evidences indicate that miRNAs are important players in resistance to radiotherapy of several tumor types. In order to understand the role of miRNAs in radioresistance of breast cancer, MCF-7 and MDA-MB-231 cell lines were exposed to fractionated doses of ionizing radiation to establish a radioresistant cell lines (MCF-7RR and MDA-MB-231RR). We analyze the miRNome in MCF-7RR and identified a set of 16 miRNAs differentially expressed. The expression of 8 miRNAs (miR-10a, miR-122, miR-222, miR-222*, miR-135b, miR-135b*, miR-196b and miR-934) were validated in MCF-7RR and MDA-MB-231RR by RT-qPCR. Interestingly, miR-122 was up-regulated (fold change 3.5 +/- 0.5) in both radioresistante cell lines. To determine the function of miR-122 in the cellular radioresistance of breast cancer, we transfected MCF-7RR and MDA-MB-231RR cells with antagomiR-122 and parental cells with mimic-miR-122. After transfection, cells were irradiated and survival was evaluated by clonogenic assays. Results showed that miR-122 significantly decrease cell survivial after treatment with ionizing irradiation. In an effort to identify novel miR-122 targets we analysed the transcriptome of the MCF-7 and MDA-MB-231 cells transfected with mimic-miR-122 by microarrays. We identified 261 and 436 genes differentially expressed in MCF-7 and MDA-MB-231 cells respectively. Of them, 68 genes in MCF-7 and 159 genes in MDA-MB-231 cells present putative miR-122-binding site in the 3′-untranslated region. Bioinformatic analyses of the gene ontology and biological processes showed that these set of genes are related to protein autophosphorylation, growth factor receptor signaling pathway, positive regulation of cell cycle, regulation of cell proliferation, regulation of cell cycle arrest, inflammatory and Immune response, regulation of extracellular matrix disassembly and positive regulation of ERK1 and ERK2 cascade. Interestingly, 9 genes (ABCA1, IL1B, IL6, IL7R, LRRFIPI1, NUDT4, CXCL10, HLA-DRA and IGLON5) were modulated in both cell lines. In conclusion, miR-122 plays an important role in radioresistance of the breast cancer cells by regulate genes involved in cell survival and inflamatory response.

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# GNAO1 and ASAH1 as probable biomarkers for pediatric ependymoma

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**Introduction.** Ependymoma is a tumor originated from migrating cells during embryogenesis. In these tumors it has been observed a methylation profile associated to malignancy due to oncogene activation and tumor suppressor genes repression. On the other hand, chromosomal alterations associated to the anatomic localization have been observed. Moreover, such alterations may be important during tumoral development as them correlate with methylation and gene expression profiles. Because of that reason it is interesting to know if a joint analysis of chromosomal alterations with the methylation and gene expression profiles can be useful to identify genes with the main importance for the tumorigenesis in ependymomas. **Methodology.** Tumoral tissue was collected from pediatric patients. For each sample CGH, methylation and gene expression microarray experiments were performed with the Agilent platform. Data were analyzed with Matlab Software and interrogating DAVID and Hippie databases. To validate microarray experiments RT-qPCR assays were performed for the genes of interest which were selected due to its biological function previously reported in databases and accordingly with their correlation observed in the 3 microarray assays. **Results.** Within the altered chromosomal regions were found *IMMT, ASAH1, ZWINT, IPO7, GNAO1* and *CISD3* genes which present a change in the methylation pattern and consistently a change in the gene expression pattern. Moreover, these genes are implicated in process like apoptosis and cell proliferation. **Conclusion.** These results suggest GNAO1 and ASAH1 are important for the tumorigenesis and may be useful as potential diagnosis biomarkers.

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# Analysis of the Genetic Diversity of *Ustilago maydis* in Mexico using Microsatellite Markers

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## Abstract

*Ustilago maydis* is a biotrophic pathogen specific for maize and teosintle, which causes the disease known as “huitlacoche” or common smut. To determine the genetic diversity and population structure of *U. maydis* 155 fungus isolates were analyzed using microsatellite markers (SSRs). Fungus tumors (corn smut) were collected from 14 different Mexican states with diverse environmental conditions. Analysis of molecular variance (AMOVA) revealed that the highest variance component is within populations (83%), and between populations (17%). The statistical $F_{ST}$ = 0.166 indicates a large genetic difference between the *U.maydis* populations analyzed. Statistical analysis shows that the number of different alleles corresponds to 3.4. Observed heterozygosity (Ho) corresponds to 0.243 and heterozygosity expected corresponds to 0.424. A dendrogram generated using the Unweighted Pair-Group Method with Arithmetic means algorithm (UPGMA) formed two groups named A and B. The first one is subdivided into four subgroups, and the second one is subdivided into two groups. Their grouping is based on SSRs and some cases based on climate characteristics. The optimal structure for *U. maydis* as Bayesian analysis and based on the value of ΔK is K = 2, which is consistent with the dendrogram. Test of Mantel indicates no genetic correlation with geographic fungus, with a value of Rxy = 0.033.

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# Ribosomal profiles and rRNA synthesis in mutants (ΔRPP1A, ΔRPP1B, ΔRPP2A and ΔRPP2B) affecting ribosomes acidic proteins of *Saccharomyces cerevisiae.*

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All cells must perform the process of protein synthesis; this synthesis is catalysed on the ribosome. The ribosome is a ribonucleoprotein with a sedimentation coefficient of 80S. It consists of two ribosomal subunits; lower or 40S or 60S and greater. In S. cerevisiae the 40S subunit is constituted by the the 18S rRNA and 33 ribosomal proteins (RPs) and the 60S subunit by rRNA's 25S (28S in higher eukaryotes), 5S 5.8S and 46 PRs. In the 60S subunit the “ribosomal stalk” is located, formed by the ribosomal protein P0 and by 4RP’s whose sizes range between 10.6 kDa and 11kDa. They have a C-terminal conserved and have the quality of having an isoelectric point less than 4, in contrast to the basic properties of other ribosomal proteins. Another particular feature is the existence of a cytoplasmic pool with which an active replacement is done. The conformation adopted in the ribosomal stalk is pentameric (RPP2A - RPP1B -P0 - RPP1A - RPP2B). PR 1 (A/B) and 2 (A/B) and the conserved region of the c-terminal of P0 are dispensable, thus allowing to obtain simple, double, triple and quadruple viable mutants. Growing this organism in liquid media is possible to characterize the lag, logarithmic and stationary growth phases.

The composition of the culture medium (Full or YEPD and minimum or SC) and temperature (20-36ºC) affect the duration of each of these phases. From yeast lysates different fractions can be obtaineed by "rate-zonal ultracentrifugation" on sucrose gradients. By this approach the ribosomal profiles were analyzed individually where populations 40S, 60S, 80S and polysomal were obtained. Using as reference the 80S fraction, normalized to a value of 1.0, different ribosomal profiles obtained can be compared, finding that ΔRPP2A mutants show an increase of 29.5 % mainly distributed in the 60S and polysomal populations. In contrast, the single mutant ΔRPP2B had a decrease of 34.4 % in the total population of the ribosomal profile. These results are consistent with the differences observed in the growth of these mutants. Complementary experiments where these simple mutations were restored, reversed the changes observed in the corresponding growth kinetics.

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# Role of the DisA-UvrABC interaction in protecting germinating/outgrowing Bacillus subtilis spores from oxidative promoted-DNA damage.

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During unpredicted periods of latency *B. subtilis* spores may accumulate DNA damage, which can be exacerbated by the entrance of water into the spore core and activation of aerobic metabolism during spore germination/outgrowth[1]. Thus, it has been shown that processing of ROS-promoted DNA damage during spore germination/outgrowth requires components of the base and nucleotide excision repair pathways as well as of RecA[2]. A recent report showed that DisA is a major component of a checkpoint mechanism when oxidative DNA damage is present in germinating/outgrowing spore DNA[3]. To discover possible interactions of DisA with components of the major repair pathways, we investigated how disruptions of distinct repair genes in DisA-deficient spores affected its return to vegetative growth. Following this approach, we found that in reference to spores bearing single *disA*, *uvrA* mutations, a double knockout *uvrA disA* strain generated spores affected in germination (as assessed by flow cytometry) and exhibiting a significant delay in spore outgrowth. Such defect was exacerbated when the mutant spores were germinated in the presence of $H_2O_2$; of note, the oxidizer agent also induced a dramatic increase in the mutation frequency to $Rif^r$ in the UvrA DisA-deficient outgrown spores. In conjunction, these results support the concept that ROS-promoted DNA damage generated during germination/outgrowth is processed by the NER system in coordination with DisA. Recently, a construct to express *uvrA* from a *dinR* (*lexA*) regulated promoter was recombined into the neutral *amyE* locus of strain *uvrA disA*. Spores of this strain are currently employed to investigate whether overexpression of *uvrA* following DNA damage suppresses the phenotypic defects observed in germinating/outgrowing *B. subtilis* spores lacking DisA and UvrA.

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# *Candida glabrata* encodes a longer variant of the mating type (*MAT*) alpha2 gene in the mating type-like *MTL3* locus

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Sexual reproduction and cell type identity in many fungi are determined by the information encoded in the mating type locus (*MAT* or *MTL* in some fungi). Mating occurs between cells of opposite mating type. In *Saccharomyces cerevisiae MAT***a** cells encode the **a1** gene (**a** information) while *MAT*alpha cells encode the alpha1 and alpha2 genes (alpha information). These genes encode transcription factors that regulate the expression of cell type-specific genes through a regulatory circuit. Cells that have mated express both types of *MAT* information, and the **a1** and alpha2 proteins interact to form the heterodimer **a1**-alpha2, which represses a set haploid specific genes, as well as some genes involved in the response to stress.

*Candida glabrata* is a fungal pathogen found as a commensal of humans, which can cause severe systemic infections in *immunocompromised* patients. *C. glabrata* is more closely related to *Saccharomyces cerevisiae* than to other pathogenic species of the genus *Candida*. It.is a haploid yeast that has not been observed to undergo sexual reproduction. However, it contains the vast majority of genes known to be required for mating.

*C. glabrata* like *S. cerevisiae* contains three *MTL* loci containing either **a** or alpha information, but in contrast to *S. cerevisiae* we discovered that *C. glabrata* contains a longer variant of the alpha2 gene in *MTL3* (which we have called alpha3), than the alpha2 gene present at *MTL1* locus,. ,Instead, in *S. cerevisiae* the alpha2 genes present at *MAT* and *HML* are identical.

Here we describe the construction of strains that simultaneously express **a1** and alpha2 or **a1** and alpha3 and we did not find any phenotype in these strains when grown under a large variety of stress and nutritional conditions. We also found evidence that *Cg***a1** and *Cg*alpha2 directly interact to form heterodimers but not with *Cg*alpha3 by Bimolecular Fluorescence Complementation assay. We will discuss the implications of our findings.

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# Mutation in *TOR* affect leaf margin and pod size in *Lotus japonicus* by altering *PIN1*, *KNOTTED1*, *KNOX1* and PHAN expression

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## ABSTRACT

In plants, leaves contribute to the largest part of the aboveground biomass. In these organs, light energy is converted into chemical energy, which plants use to grow and complete their life cycle by setting the pods and seeds. So far, studies leading to the identification of numerous genes that contributes to the final size of leaves have been identified in *Arabidopsis viz.*, *PIN1* and *KNOX1* are indicators of leaf initiation, while Growth Regulating Factor 5 (*GRF5*) control leaf expansion and maturation; besides, recent research has identified new players such as *APUM23*, known to specify leaf polarity. On the other hand PHANTASTICA (PHAN) pays multiple roles including the regulation of leaf size, shape and pod size. However, the molecular genetic basis of the relationship between leaf development and pods or fruits is poorly understood. Here we show the phenotype of *Lotus japonicus* mutant for target of rapamycin (*TOR*). TOR is a Ser/Thr protein kinase that regulates nutrient-sensing, cell proliferation, growth and cell death in diverse eukaryotes. We observed the irregular leaf margins when TOR is mutated compared to the wild-type. The RT-qPCR analysis of mutants reveals downregualtion of different isoforms of PIN1, KNOTTED1, KNOX1 and GRF5. However, the expression of APUM23 was not affected. Furthermore, the mature pods of the TOR mutants were significantly small in the size and it also shows downregulation of PHAN expression compared to controls. Our results suggest that TOR acts as an upstream gene that regulates the transcripts of several genes that determine the leaf shape and pod development in *L. japonicus*. This work is partially supported by PAPIIT (DGAPA-UNAM) grant no. IA205115 to MK.A. and CONACYT-240614 to M.L.

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# Influence of hydrogen peroxide ($H_2O_2$) in the regulation of the Pht cluster of *Pseudomonas syringae* pv. phaseolicola NPS3121

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*Pseudomonas syringae* pv. phaseolicola is the causal agent of the disease in common bean *(Phaseolus vulgaris* L.) known as "Halo Blight", which is characterized by the presence of chlorotic halo in leaves. This chlorotic halo appears as a result of a toxin produced by the bacteria known as phaseolotoxin. Phaseolotoxin [$N^{\delta}$(N-sulfodiaminophosphinyl)-ornithyl-alanyl-homoarginine] is a extracellular non-host specific toxin and inducing chlorosis whose production is low temperatures (18°C) dependent. Recent studies have identified genes necessary for the synthesis of this compound in a chromosomal region known as "Pht cluster", which is composed of 23 genes, arranged in five transcriptional units. So far, the regulatory pathways involved in the synthesis of phaseolotoxin have not been elucidated. Recent analyses of transcriptomic have suggested that mechanisms of oxidative stress response, particularly regulatory pathways, could to be coordinating the physiology of the bacterium at low temperatures. So the aim of this work was to determine the effect of the oxidative stress inducer agent, hydrogen peroxide, on the expression of the Pht cluster genes involved in the phaseolotoxin synthesis in *P. syringae* pv. phaseolicola NPS3121. Analyses of expression of the Pht cluster genes were performed by RT-PCR technique in conditions of presence of hydrogen peroxide ($H_2O_2$, 1mM and 2.5 mM). The results demonstrated that hydrogen peroxide caused an increase in intracellular levels of reactive oxygen species (ROS). Likewise the results demonstrated that in these oxidative stress conditions the expression of the Pht cluster genes is affected, being this effect concentration-dependent. The results of this work suggest that the Pht cluster genes are targets of the regulatory pathways of oxidative stress response.

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# Role of Bdp1 in RNA Polymerase III transcription in *Leishmania major*

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The protozoan parasite *Leishmania major* causes leishmaniasis in humans. The parasite is also relevant for showing atypical mechanisms of genetic expression, including polycistronic transcription. Our group is interested in the analysis of transcription by RNA polymerase III (Pol III), involved in the synthesis of essential RNA molecules, such as 5S ribosomal RNA (rRNA) and transfer RNAs (tRNAs). TFIIIB is a transcription factor required for Pol III function. It is composed of three subunits: Bdp1, Brf1 and TBP. Bdp1, characterized by the presence of a highly conserved SANT domain, participates in the formation of the preinitiation complex. In the *L. major* genome, we have identified an orthologue of Bdp1 (LmBdp1) that possesses the characteristic SANT domain. By performing RT-PCR analysis we showed that the LmBdp1 mRNA has long 3´ and 5´-UTR regions of ~1100 bases. We determined that the half-life of the mRNA is ~50 min in mid-log phase cells and ~2 hours in stationary-phase cells. Therefore, sequences present in the UTRs might be involved in stabilization of the mRNA in stationary-phase cells. To show the participation of LmBdp1 in Pol III transcription, we have generated null mutants by homologous recombination. A single knock-out cell line for LmBdp1 was obtained by transfecting *L. major* with a vector that contains the puromycin (pac) resistant marker, and clones were selected in the presence of pac. The double knock-out cell line was generated by transfecting the single knock-out cell line with a vector that possesses the hygromycin (hyg) gene. Southern-blot analysis confirmed the replacement of one LmBdp1 allele by the pac gene and the other one by the hyg gene. However, a third copy of the LmBdp1 gene was generated in the double knock-out cell line. This result indicates that LmBdp1 is essential for cell growth and that the knock-out cell line had to generate a third copy of the gene in order to survive. Interestingly, this cell line grows slower than wild type cells, presumably by a reduction in Pol III transcription. We are currently performing nuclear run-on assays to prove this hypothesis.

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# Role of *GRHd3* Genetic Variant in Reducing Weight in Morbidly Obesity Patients

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**Introduction**: In Mexico, morbid obesity has increased to reach 3% of the population. In order to conduct bariatric surgery, patients should reduce 10% of their weight excess. *GHRd3* gene variant encodes an isoform of growth hormone receptor without exon 3 that has been described with greater affinity for hormone. The aim of this study was to determine the association of genetic variation GHRd3 with reduction of body weight in morbidly obese patients.

**Methods**: Prospective cohort of morbidly obese patients treated at Morbid Obesity Multidisciplinary Clinic from Hospital de Especialidades, CMN Siglo XXI. Patients with BMI ≥ 40 kg/m$^2$, without relationship with another participant and ancestry at least three generations in Mexico, were invited to participate. Final measurement was carried out at six months of the pre-consultation. Patients with 10% reduction of overweight were considered with weight loss. Genotyping was performed by PCR multiplex using primers previously described (Genbank AF155912 and AF210633). Amplicons were analyzed by electrophoresis in 2% agarose gels. Hardy-Weinberg equilibrium was calculated. To determine the *GHRd3* association a dominant allele model was constructed to calculated relative risks with confidence intervals at 95%.

**Results**: One hundred ninety-four patients were included in the study. Most were women (78%). Genotype frequencies were fl/fl (32.5%), fl/d3 (50.5%) and d3/d3 (17%); allele frequency was 42% for d3, no deviations from Hardy-Weinberg equilibrium were observed (p> 0.05). No association between genetic variation *GHRd3* and reduced body weight at six months follow-up was found (RR = 0.853, 95% CI [0556-1307]).

**Conclusion**: GHRd3 genetic variant is not associated with weight reduction in morbid obese patients.

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# Analysis of functional status of c-Met receptor and its relationship with stemness and invasive capacity of a small subpopulation derived from cell lines of gastric cancer.

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Cancer Stem Cells (CSC) are limited subpopulation of stem cells with tumorigenic capacity, due to their ability for self-renewal, proliferation with a specific potential cell differentiation that results in heterogeneous cell populations that rinse a tumor (1). Activation of the cell signal transduction pathway HGF/c-Met in several carcinomas has been linked to invasive growth phenomenon, in which some tumor cells migrate and invade other tissues by the epithelial-mesenchymal transition (EMT) (2). Moreover, activation of various signaling pathways has been associated with the stemness of some cancer cells, for example, the activation of c-Met receptor induces stem-like phenotype in prostate cancer (3). Since, HGF/c-Met signal pathway activation could be crucial for stemness, the aim of this study is to evaluate the activation of c-Met receptor in Gastric Cancer Stem Cells (GCSC) enriched culture derived from gastric cancer cell lines. Results: We standardize the conditions to form spheres derived from AGS and NCI-N87 gastric cancer cell lines to enrich GCSC population. Flow cytometry analysis shown an increase of the percentage of $CD44^{high}$ cells in gastric spheres used as GCSC marker. We observed an activation of c-Met in GCSC enriched culture due to the increase of phosphorylation of this receptor. Also, observed up-regulation of the stemness transcription factors, Oct4 and Nanog in gastric spheres compare to monolayer cultures. The specific inhibition of phosphorylation of c-Met receptor by PHA665752, decreased Oct4 and Nanog proteins in gastric sphere cultures. Moreover, through clonogenic assays, we observed that inactivation of c-Met receptor results in a smaller number of colonies compare to cells non-inhibitor cell culture conditions, while colonies without treatment showed “hummingbird phenotype”, that is related to an invasive phenotype. In conclusion, receptor phosphorylation might be involved in stemness and also may be in the invasive capacity of a specific GCSC subpopulation.

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# MicroRNAs-mediated regulation of the tumor suppressor Merlin in response to inflammatory signals.

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Abstract

Numerous evidences indicate that chronic inflammation promotes tumors progression. In some cases, inflammatory conditions are present before a malignant change occurs. But in other case, an oncogenic change induces an inflammatory microenvironment that promotes tumors progression. Nonetheless, the molecular pathways activated by inflammation that promote cancer development are not totally elucidated. MicroRNAs are approximately 22 nucleotide single-stranded RNA that regulate protein levels by selective binding to specific sites at the 3'-untranslated regions of their target messenger RNA (mRNA). Different cellular functions are regulated by specific microRNAs expression patterns. Interestingly, chronic inflammation alters microRNAs expression, thus favoring the development of different diseases, including carcinogenesis. Merlin, the protein product of NF2 gene, acts as tumor suppressor regulating cell proliferation, migration and invasion. Loss of NF2 tumor suppressor gene is involved in the development of neurofibromatosis type 2 syndrome. Inactivation of NF2 gene also has been associated with other tumors as malignant mesothelioma, osteosarcomas, fibrosarcoma and hepatocellular carcinoma. In addition to Merlin negative regulation by phosphorylation, we have recently shown that Merlin is also negatively regulated by microRNAs, including miR-146, mir-7, mir-32 and mir-25. Down regulation of Merlin by microRNAs 146 and 7 in A549 cells enhanced proliferation, cell migration and tumor formation. According with the fact that pro-inflammatory signals induce miR-146 and mir-7 expression in an NFkB-dependent manner, here we show that proinflammatory conditions promote expression of miR-146, mir-7, and mir-32 in A549 cells. These results correlate with a downregulation of Merlin protein levels and increase of cell migration in A549 cells. We propose that microRNAs induced by proinflammatory conditions promote cell transformation by targeting Merlin. This work is partially supported by grants from DAGPA/UNAM (IN212316 and IN-213316)

Keywords: microRNAs, tumor suppressor, Merlin, inflammation, cancer

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# Role of translation initiation factors eIF4E and eIFiso4E during cold stress response in *Arabidopsis thaliana.*

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Plant abiotic stress response is a complex process that occurs when plants are exposed to unfavorable conditions during growth, such as high or low temperature, drought, elevated ground salinity and others. This process involves a specific gene expression patterns as well as tight regulation of protein synthesis to increase plant resistance and survival capacity. Translation regulation emerges as important step in stress response. There is evidence of general translation inhibition under stress, but particular protein production remains active for homeostasis maintenance. The mechanisms involved in translation specificity under stress are poorly understood. In our group we focus on the role of translation initiation factors belonging to the 4E family (eIF4E) in *Arabidopsis thaliana* response to freezing temperature. These factors are major players in protein synthesis regulation in other organisms. They recognize the 5' end cap structure on mRNAs and recruit the rest of the translational machinery. In *Arabidopsis* there are two isoforms termed eIF4E-1 and eIF4E-2 or eIFiso4E that under normal growth conditions apparently have redundant functions. However, experimental evidence pinpointed certain isoform preference in selection of mRNAs encoding stress response proteins. Whether there is specialization of these factors in abiotic response as reported for other organisms remains an open question. Here we tested the expression of these factors after cold treatment and found that their production is upregulated under this condition. On the other hand, phenotypic analyses of plants overexpressing either eIF4E (*35S:eIF4E*) or eIFiso4E (*35S:eIFiso4E*) compared with wild type plants showed increased resistance after a freezing treatment. Interestingly, the absence of eIFiso4E (*AteIFiso4E-1*) has a detrimental effect on plant response to this type of stress even in the presence of eIF4E suggesting that the role of these isoforms in freezing tolerance is not completely redundant. In order to investigate at molecular level the specialized action of this factors during stress response, we have selected a group of cold activated genes proposed as preferentially translated by each one of these factors to analyze their expression pattern in the mutant lines mentioned before. We hypothesize that selective translation of stress responsive mRNAs by eIF4E isoforms might underlay their specialized function in cold temperature plant response.

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# Transcriptional changes occurring in nitrogen fixation by an endosymbiotic bacteria in the basidiomycota fungus *Ustilago maydis*.

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Understanding how individuals belonging to different domains of life communicate via molecular signals is an interesting trend topic in the field of fungi-bacteria and plant interaction. Elucidate communication strategies and discovery of the fundamental molecular mechanisms of how endosymbionts and pathogens fungi survived extreme conditions of food scarcity is important. To grow and develop normally, non-parasitic fungi must obtain sufficient carbon sources, and either inorganic or organic nitrogen from the media. In fungi, nitrogen is required for the synthesis of proteins, nucleic acids, amino acids, chitin and so on.

The fungus *Ustilago maydis*, a plant parasitic basidiomycota causing corn smut disease, is able to survive and grow in nitrogen lacking media. We have recently reported the interaction of endocytic bacterium with *U. maydis,* that provide the fungus with the capacity to fix atmospheric $N_2$. Possibly the fungus and the bacterium exchange signals that, besides nitrogen fixation, may regulate each other´s metabolism, physiology and development; all this to guarantee their interaction. In this concern, our research group seeks to elucidate at the transcriptomic level, how the transcriptional regulation in a medium lacking fixed $N_2$ takes place in the fungus. We demonstrated throughout FISH methodology the endosymbiont presence, and through sequencing of 16S rRNA PCR and NifH genes products we confirmed that the endosymbiont corresponded to a *Bacillus sp.* The transcriptomic analysis of the fungus showed that the regulated genes during $N_2$ fixation were grouped into several functional categories. Metabolism, cellular transport, interaction with the environment, among others were the highest representative categories. Using B2GO software, we found that, anion transmembrane transport; ammonium transmembrane transport and other nitrogen compounds transport were enriched in the processes in the fungal cell. Transcriptomic analyses also indicated that differential regulations of genes involved in various cellular processes in response to adaptation of nitrogen were missing. This implies that the absence of fixed nitrogen activates a set of genes to lead the input of the nitrogen provided by nitrogen fixation conducted by the endosymbiont to the fungal. This mode of symbiosis is favorable as it provides adaptation to the stress condition.

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# *Ustilago maydis* gene *NRG1* identification and deletion.

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The Basidiomycete *Ustilago maydis* presents an excellent pH adaptative behavior, since it can grow at externals pH ranging from 3 to 10. Adaptative strategies involve morphology changes and expression of pH responsive genes. Among these, the alkaline response transcription factor Rim101 has been previously described and a considerable percentage of the genes regulated by this transcription factor present a putative Nrg1 binding site in their promoter region (1). Nrg1 has being described in *Sacharomyces cerevisiae* as a GAL and SUC2 repressor in glucose presence and it is associated with $Na^+$ and $Li^+$ high concentrations tolerance (2).

In this work we present the identification of the possible *nrg1* ORF in the *U. maydis* genome. *In silico* analysis reveals the presence of two zinc finger typical domains conserved in other fungal putative homologs. Also a carboxine resistance interruption cassette was constructed and used to interrupt the *U. maydis* gene by the “Double Joint” strategy (3). UmNrg1 deletion was probed by PCR with internal nrg1 ORF oligonucleotides in *a2b2* genetic background. Further mutant´s phenotypic analysis would be done in order to elucidate UmNrg1 specific function not only in the fungal general metabolism, also in pH change adaptation strategies associated to Rim101.

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# Deleting a gene in a hard to transform yeast. The story of *Debaryomyces hansenii.*

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*Debaryomyces hansenii* has been studied for its capability to adapt to saline stress. It was originally isolated from marine water, but it can also be found in salty foods. It can accumulate high $Na^+$ concentrations, and its adaptation has been attributed to some improved enzymes, different metabolic routes, or a combination of processes converging to cope with saline stress.

The HOG (high osmolarity glycerol) pathway mediates a significant part of the response of yeast cells to a hyperosmotic shock, since it is required for the stimulated expression of more than 100 genes. The osmo-induced genes include GPD1 and GPP2, which encode enzymes involved in the production of glycerol, the main osmolyte accumulated by yeast cells.

It is of our interest to know the participation of the general Hog1 pathway in the saline resistance of *D. hansenii*, but this yeast is hard to transform. Several groups of colleagues have tried to delete some genes without success. We contacted Dr. Papon in France since he and his group have designed some plasmids to transform yeast from the CTG clade.

The yeasts from the CTG clade translate the codon CTG in serine instead of leucine. The reassignment of the CUG codon from Leu to Ser occurs in at least 75 *Candida* species and in *Pichia stipitis*, *D. hansenii* and *Lodderomyces elongisporus*.

Using the p244 plasmid which encodes a *SAT1* gene for resistance to nourseothricine and a *yeYFP1* gene for yellow fluorescent protein with optimized codons for expression in *D. hansenii*, we succeeded in obtaining a *hog1* mutant of this yeast.

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# *Ustilago maydis* metacaspase is involved in response to stress

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Metacaspases are cysteine-dependent proteases found in protozoa, fungi and plants, that cleave their targets after Arg or Lys residues. These proteases are involved in the breakdown of complex protein peptide bonds during the apoptosis process. The number of metacaspase genes in the genomes of different organisms varies considerably, for example *Saccharomyces cerevisiae* has a single metacaspase (Yca1) required for oxidative, salt, and osmotic stress-induced cell death, *Candida albicans* also has a single metacaspase gene (CaMCA1) required for oxidative-induced. In contrast, *Aspergillus fumigatus* contains two metacaspases involved in the resistance to oxidative stress.

In this study we proceeded to determine the role of metacaspase from the dimorphic Basidiomycota fungus *Ustilago maydis*, the causal agent of corn smut or Huitlacoche in maize (*Zea mays*). By means of *in silico* analysis we identified the unique *MCA1* gene (UMAG_01408). This gene was mutated in the solopathogenic merodiploid SG200 strain by the DelsGate technology. Gene deletion was verified by PCR, RT-PCR and Southern blot.

In the case of the sexually compatible strains, FB1 (a1b1) and FB2 (a2b2), mutants were obtained by double homologous recombination using, as selection marker, the hygromycin resistance cassette ($Hyg^R$).

Growth of the mutants in different carbon sources was not affected in comparison with the parental wild type strains. On the other hand, thus Δ*mca1* mutants were more resistant than wild type to the stressants dithiotreitol (DTT), tert-butyl hydroperoxide (tBOOH), LiCl, $CdCl_2$, $CoCd_2$, but were more sensitive to the stressant congo red. Interestingly, they were more resistant to acetic acid, and $H_2O_2$, known apoptosis inducers.

*Ustilago maydis* Δ*mca1* mutants were complemented with the whole *MCA1* gene from *U. maydis*, including the promoter and terminator regions, inserted at the IP (succinate dehydrogenase *locus*), using carboxin resistance as selective marker. Carboxin-resistant transformants were recovered and the complementation was confirmed by PCR and used for phenotypic characterization. As expected, the wild-type phenotype was recovered in the complemented strains.

In summary, our data are evidence that metacaspase is involved in the response to some stress conditions, and possibly somehow in apoptosis in *U. maydis*.

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# Feronia gene has a significant role in the *Phaseolus vulgaris*- rhizobia symbiotic interaction

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FERONIA (FER) is a plant membrane receptor like kinase, belonging to the CrRLK1l family. FER participates in a variety of plant processes such as growth, fertility, hormone signaling and polar growth, among others (1-3). To analyze the functional role of *Phaseolus vulgaris* FER while in symbiosis with the soil bacteria *Rhizobium tropici*, a reverse genetics approach was used. To do this, first a bioinformatic analysis was performed to determine if FERONIA constitutes a gene family in bean. The results obtained indicate that there are two FER genes in the *P. vulgaris* genome data base (http:phytozome.jgi.doe.gov/), that we named *PvFER1* and *PvFER2*. Both genes are highly transcribed in root hairs, as compared to root apex and stripped roots. *PvFER1* transcript in root hairs is accumulated ten times higher than *PvFER2*; hence the former was selected for further characterization. The transcript accumulation profile of *PvFER1* was analyzed in *P. vulgaris* roots before and after inoculation with *Rhizobium tropici*, that is its natural microsimbiont. Different *PvFER1* transcript accumulation profiles were observed in roots from plants inoculated with *R. tropici* at different time points (3, 5, 7, 9 and 15 days post-inoculation) when compared to non-inoculated roots, suggesting a role of this gene in the nodulation process. Loss-of-function of *PvFER1* in transgenic roots inoculated with *R. tropici*, showed a slightly reduced number of nodules with smaller size, although no differences were found in nitrogen fixing levels, when compared to control transgenic roots. On the other hand, *PvFER1* gain-of-function in transgenic inoculated roots displayed approximately 20% more nodules, though its size is diminished; nitrogen fixation levels remain unchanged, as compared to the controls. These data, taken together prompted us to propose that *PvFER1* has a significant role in nodulation in *P. vulgaris*, specifically during the early stages of this process.

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# Regulation of expression of the *nrdEF* operon encoding the class 1b ribonucleotide reductase in *Bacillus subtilis*

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Ribonucleotide reductase (RNR) catalyzes the reduction of nucleotide triphosphates (NTPs) to deoxyribonucleotide triphosphates (dNTPs), essential precursors for replication. RNRs are subjected to extensive allosteric regulation in most organisms. However, the intracellular amount of these enzymes is also regulated at transcriptional level; such control is of particular relevance in bacterial species possessing different types of RNRs, including *E. coli* and *Pseudomonas aeuroginosa*. *Bacillus subtilis* relies on a single 1b-class RNR composed of two homodimeric $\alpha_2/\beta_2$ subunits that are encoded by the second and third cistrons of the *nrdIEF* operon.

In this work, we engineered a *B. subtilis* strains carrying a transcriptional *in frame nrdEF-lacZ* fusion to analyze the expression of the RNR-encoding genes during the life cycle of this microorganism as well as its possible induction by transcription factors controlling stress responses in this bacterium.

Results showed that *B. subtilis* constitutively expressed high levels of the reporter *lacZ* gene in exponentially growing cells of the strain *nrdEF-lacZ* and that such activity decreased during the transition and stationary phases of growth. Of note, the levels of the *nrdEF-lacZ* fusion increased significantly following disruption of *ytcG*, which encodes the putative NrdR repressor. Furthermore, the *nrdEF* directed β-galactosidase levels increased significantly in genetic backgrounds deficient for the transcriptional regulators PerR and $\sigma^B$, which control the oxidative and general stress responses in *B. subtilis*, respectively.

Therefore, metabolic conditions that promote nutritional and genotoxic stresses may directly or indirectly activate *nrdEF* expression in this bacterium, possibly to provide dNTPs required for mutagenesis and/or DNA repair.

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# CRISPR/Cas9 Mutagenesis of *Sporothrix schenckii RmlD*

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Sporotrichosis is a subcutaneous mycosis caused by the dimorphic fungus *Sporothrix schenckii*. The disease origins from a cutaneous inoculation, when *Sporothrix* conidia enter into subcutaneous tissues. In order to colonize the host tissues the pathogen has to have the capacity to adhere to them. The cell wall sugar polymers and proteins of *Sporothrix* act as pathogen-associated molecular patterns (PAMPs), these PAMPs bind to the pattern recognition receptors (PRRs) on the innate immune cells, triggering the signals to activate the host immune system response. A well known cell wall component of *S. schenckii* is the peptidorhamnomannan. The yeast cell wall peptidorhamnomannan is composed of 33.5% rhamnose, 57% mannose and 14.2% protein. The L-rhamnose is synthesized in similar ways in bacteria, plants and fungi. Its precursor dTDP-L-rhamnose is synthesized from α-D-glucose-1-phosphate and dTTP in a pathway that requires four different enzymes: RmlA, RmlB, RmlC and RmlD. RmlD catalyzes the final step in this pathway converting dTDP-6-deoxy-L-*lyxo*-4-hexulose to dTDP-L-rhamnose. Using bioinformatics approach we identified the putative orthologue of *RmlD* in *S. schenckii,* which encodes for a dTDP-4-deoxyrhamnose reductase. The CRISPR/Cas9 system is a simple and efficient tool for genome editing that has experienced a fast progress in its technology and applicability. To demonstrate the function of *S. schenckii RmlD* we applied this system to generate mutants lacking RmlD.

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# Study of genetic variability in virulence factors among strains of *Avibacterium paragallinarum*

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The bacteria *Avibacterium paragallinarum* is the etiological agent of infectious coryza call, a disease upper respiratory tract of birds, It is characterized by producing nasal discharge, sneezing and facial swelling. Complicated or infectious form does cause death. Its distribution is worldwide and causes significant economic losses to the poultry industry due to stunting, weightloss and decreased egg production (Blackall *et al*., 2003). This bacterium several virulence factors among which are known toxins, proteases, hemagglutinin and other indirect association are alleged virulence factors.

In this work the bioinformatic study of 5 virulence genes *Avibacterium paragallinarum* was to determine the genetic variability that may occur in these genes, in order to understand the differences between different isolates virulence. Of these five virulence genes , 2 have already been reported as encoding virulence factors *iga* y *rtx*, and the other 3 possible genes virulence genes *rpo*D, *ssb*, *gln*A1, suggested by Requena D *et al* 2013. The analysis was made from 4 known genomes of *Avibacterium paragallinarum* strains ; CL , 72, JF4211 and 221, sequences where the five genes were obtained to be analyzed individually. For comparison , alignment and phylogram the sequence of a housekeeping gene (*adk*) *E. coli* was used as root. This result was compared as another phylogenetic analysis done with housekeeping genes (adenilate kinase, fumaratehydratase, DNA gyrase, Isocitrate dehydrogenase, malate dehydrogenase, adenylosuccinate synthetase y RecA protein) of the 4 strains of *Avibacterium paragallinarum*. Strains diverged along virulence sequences selected from 3 to 5%, using CLUSTALX y MEGA5. The data show little evolution of virulence factors in *Avibacterium paragallinarum*. At this time and is being done assessing the usefulness of target sequences chosen as a comparative tool to investigate the pathogenicity and virulence of *Avibacterium paragallinarum*.

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# Sum1 as a virulence factor in *Candida glabrata*

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**Abstract**

*Candida glabrata* is a commensal yeast that can act as an opportunistic pathogen in immunocompromised patients. In the last years, there has been an increased incidence of *C. glabrata* as etiologic agent of candidemia. In *C. glabrata*, the expression of drug efflux pumps is one of the strategies to adapt to hostile environments. In *C. glabrata*, oxidative stress response and multidrug resistance are negatively regulated by the Hst1-Sum1 complex. Hst1 is a histone deacetylase that uses $NAD^+$ as cofactor and Sum1 is a DNA-binding protein. Deletion of *HST1* or *SUM1* (*hst1*Δ or *sum1*Δ) decreases susceptibility of *C. glabrata* to fluconazol (FLC) through the increase in expression of *PDR1* (transcription factor) and *CDR1* (drug efflux pump). However, this decreased susceptibility of *C. glabrata hst1*Δ to FLC depends on the growth media. *C. glabrata hst1*Δ is susceptible to FLC (32µg/ml) when grown in RPMI-1640 or casamino-acids (CAA) media but it is resistant when grown in rich media (YPD). Data obtained by quantitative PCR-based analysis of *CDR1* expression in the wild-type and *hst1*Δ strains of *C. glabrata* is consistent with the genetic data. Analysis revealed that cells grown in RPMI-1640 media have a diminished *CDR1* expression, even in the absence of Hst1. We evaluated if there are diffusible elements in the growth media that could be modulating the FLC resistance/susceptibility. Conditioned media (CAA or YPD) and stationary phase cells from *C. glabrata* wild-type and *C. glabrata hst1*Δ cultures were mixed and grown in CAA or YPD at different concentrations of FLC. These results showed that there are no diffusible molecules responsible for modulating the resistance or sensitivity to FLC in wild-type and *hst1*Δ strains. Regardless of the combination of media used for grow, the resistance/sensitivity to FLC (32µg / ml) of the *hst1*Δ strain is determined directly by the end growth media.

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# Construction of vectors for *MTL* gene expression in *Candida glabrata*

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KEY WORDS. Inducible translational fusions, *Candida glabrata*, MTL.

Sexual reproduction in most fungi is controlled by genes encoded in the *MAT* locus (Mating Type Locus) or *MTL* (Mating-Type Like) in other fungi. In *Saccharomyces cerevisiae* these genes encode transcription factors involved in the establishment of the cell-type identity and regulation of mating. *Candida glabrata* is a haploid and asexual yeast that is an opportunistic pathogen of humans. However, it has orthologous genes to those that regulate mating in *S. cerevisiae,* called **a1**, alpha1 and alpha2 encoded in the *MTL* loci. The fact that *C. glabrata* has preserved these genes, even though there is no experimental evidence of sexual reproduction, raises the question of what is the role of these genes in this organism.

To elucidate the function of these genes, we have generated four different replicative recipient vectors in *C. glabrata*, that allow us to generate translational fusions with two different epitopes (Flag or c-Myc) or two fluorophores (mCherry or YFP), at either the C or N-termini of the genes of interest. These plasmids are replicative in *C. glabrata* and have different selection markers to allow for selection of two plasmids in a single cell, and coexpression of two different tagged proteins.

In addition the vectors contain one of two inducible promoters: the promoter of either *MET3* or *MT1* genes, so it is possible to tag the genes of interest at either end and induce the fusions by growing in specific media. We generated different translational fusions of the *MTL* genes at the carboxy and amino termini with both fluorophores and epitopes used in this work. We will use these fusions to identify the cellular localization of these proteins (in the case of genes tagged with fluorophores), as well as studies of DNA-protein interaction (ChIP, ChIP-seq) and protein-protein interactions by co-expression of different genes in the same cell (CoIP).

In parallel, we have cloned each wild-type gene (with no epitope or fluorophore fusion) under the inducible promoter $P_{MT1}$ to study the effect of the over-expression of these genes.

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# Role of the Oxidative Stress in the expression of the Pht cluster genes involved in the phaseolotoxin synthesis in P. *Syringae* pv. Phaseolicola NPS3121.

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# mRNA expression of the glutathione related genes in cultured HeLa cells treated with valproic acid.

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It has been indicated that epigenetic changes are involved in carcinogenesis, which unlike genetic alterations, are reversible indeed. The main epigenetic mechanisms whereby genetic changes are achieved include processes such as “CpG islands” hypo and hyper methylation, post translational histone modifications and downregulation of the non-coding miRNAs expression. The study and development of drugs capable of modify these variables (known as epigenetic drugs) have been promoted by these findings.

On the other hand, the biggest challenge to overcome within the anti-cancer therapeutics is the development of multidrug resistance by malignant cells. The resistance shows up through many mechanisms all known as the MDR phenotype, this phenotype includes features like drug uptake decrease, drug efflux increase by ABC transporters, detoxyfication systems activation (phase II metabolism, glutathione system), etc. This study focuses on the relation between ABC transporters expression in cultured HeLa cells treated with VPA (valproic acid, drug which is now studied by its epigenetic variables modifying properties) and the detoxification by glutathione system, since it is the main non-proteinic thiol present in all tissues, that has three principal functions: alternative membrane transport of aminoacids as γ-glutamil amino acids, antioxidative defense agains ROS toxicity and xenobiotics detoxification by means of phase II conjugation reactions. There is evidence of multiple pathological conditions due to GSH system deregulation, among which is the MDR phenotype development by malignant cells. It has been reported that the ABC transporters overexpression in HeLa cells could induce an upregulation in its intracellular GSH concentration which increases even more its multidrug resistance capacity.

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# Effect of the E6 and E7 oncoproteins of the HPV18 in the genes expression associated whit the glycosylation.

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The Infection with certain genotypes of Human Papillomavirus (HPV), as HPV-16 and -18 is required for the development of premalignant lesions and cervical cancer. The HPV genome encodes two oncoproteins E6 and E7 that are essential for the maintenance of malignant transformation and evasion of apoptosis by their interactions with various target proteins as p53 and Rb respectively. The glycosylation is a process closely linked to carcinogenesis. The glycans found at the cell surface have essential biological functions and changes in expression of these, they have been associated with alterations in gene expression of enzymes that involved in their synthesis. The aim of our study is to determine whether the E6 and E7 oncoproteins of HPV18 have any effect on the expression of glyco-genes.

In HeLa cells (HPV-18) with stable silencing of the E6 and E7 oncogene (shE6/E7) and control HeLa (shControl) Cells donated by doctors Aguilar-Lemarroy A and Jave-Suárez L (from CIBO, IMSS), clonal selection was made. The level of silencing in different clones was assessed by qPCR and Western blot. A microarray expression was performed and identified genes which modify their expression by the presence of E6 and E7 oncoproteins. We perform bioinformatic analysis to search for disorders related to glycosylation were used different softwares available in the network.

One clone was selected whose level of silencing E6 / E7 was the lowest. Microarrays expression were performed of 32K, Genes with a Z-score ≥2 and ≤2 were considered with altered expression. We Identified 544 genes downregulated and 613 genes upregulated, and 18 genes that are associated with glycosylation (nine downregulated and nine upregulated). we build networks of protein-protein interaction to observe the interactions of the glyco-genes altered with various cellular proteins and to know which pathways are affected, we Identified three networks, one downregulated that is associated whit glycosylation of protein that contain epidermal growth Factor (EGF-like) repeated, and two neworks upregulated one that are associated in the synthesis of Tn and T antigen (Mucin-type O-glycosylation) and other associated in the synthesis of galactosylceramide. Some of these genes related to glycosylation have been reported with aberrant expression in different types of cancer.

The E6 and E7 oncoproteins of the HPV18 could be regulating the expression of genes related to glycosylation. Alterations in the glycosylation may confer different behaviors tumor cells like metastatic capability, apoptosis resistance, deregulation of cell adhesion and cell-cell communication and deregulation of signaling pathways.

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# Characterization of the cellular receptors diversity for astrovirus

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Astrovirus is an important ethiological agent of gastrointestinal diseases in children worldwide. Currently eight different human serotypes have been reported.The human astrovirus (HastV) is a non-enveloped virus, its genome consists of a single strand of positive RNA with 3 ORF's: 1a, 1b and 2. ORF2 encodes the capsid protein (CP). New synthesized HAstV assembles into inmature particles inside cell, the capsid protein undergoes a maturation process via proteolytic cleavage steps that are required for virus releasement and infectivity. When the maturation of the virus is complete, viral capsids are composed by three predominant proteins, VP34 at core and VP25 and VP27 at spikes. The study of the crystallographic structure of the capsid of HAstV serotype 8 suggest that the recognition site of this virus its localized in the spikes, due to the identification of a characteristic carbohydrate recognition domain in its structure.The region ORF2, encoding the spike, is hypervariable; so it is probable that the various serotypes recognize different cell receptors. This could be the reason of the different cell tropism present between serotypes. We have realized Far Western techniques, with purified membranes of Caco-2 and HAstV serotype 8 in order to identify possible cell receptors. Until now, we have detected 2 bands of possible receptors, one at 70 kDa and another at 73 kDa. The idefication will been completed using mass spectrometry.

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# Dynamics of antagonistic interactions in an invasive network of bacteria

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Competition has been considerate as one of the main operators of the assembly of the bacterial communities. However, it is largely unknown how bacteria detect the presence of a competitor, the specificity of the response and how fast this reaction is. To determine how bacteria sense and respond to an invasion we evaluated interactions with three strains with different roles in an antagonistic network (strain A, non-antagonist and resistant; strain B, antagonist and resistant; strain C, non-antagonist and non-resistant). The bacterial strains used in this work were isolated form sediment samples of the Churince system in Cuatrocienegas, Coahuila; these bacteria belong to the *Bacillus* genus. The experiments were made on semisolid medium to simulate the structure of a community in the sediment. So far, we have shown that strains with different roles in the network survive together despite the antagonism among them. We hypothesized that this co-colonization is possible through the formation of "bacterial patches" in which the sensitive bacteria or the non-antagonist growth isolates and avoids the antagonist. Our results suggest that the response is fast and allows the sensing of bacteria among themselves. To determine the time and frequency-dependence of the reply of the strains during the interaction with a competitor, we will perform a transcriptomic analysis on semisolid medium. To establish frequency-dependence and a time kinetics, we cultured together the confronting strains in liquid medium with a proportion of 75% of the sensitive bacteria and 25% of the antagonist or resistant, the sensitive bacteria died in approximately 30 minutes in the presence of the antagonist and survived in the presence of the resistant. q-PCR will be used to determine the presence of each bacteria in semisolid medium and quantify its proportion. The data obtained so far allow us to hypothesize that bacteria 'have a plan' to respond to the aggression of other bacteria, and are not improvising a non-specific response.

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# The c-di-GMP protein MucR is necessary for cyst formation but not for alginate synthesis in *Azotobacter vinelandii*

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*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 2013); 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 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 we found that MucR was essential for the formation of desiccation resistant cyst in an alginate-independent manner.

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# Microbial consortia with different biochemical capabilities for hydrocarbons degradation

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The presence of oil derivatives, as a fuel, plastics, etc., in the environment is an important pollution increasing problem. For that reason, our research group has focused in the isolation of bacteria, yeast and fungi in the borders of Lerma River in Salamanca, Guanajuato, close to the oil refinery, with the ability to use hydrocarbons as an only carbon source. These diversity of wild type strains isolated from this polluted environment, are part of an ecological habitat, and they have to work together as a consortia, in order to survive. This concept had been used by different research groups, which had purpose the use of microbial consortia to improve hydrocarbon degradation, using their different biochemical capabilities. In this work, we study the metabolic capability for degrading aromatic compounds, and genes related to polycyclic aromatic hydrocarbon degradation pathway, and also the ability to growth together. In order to design microbial consortia able to bioremediate water sources, polluted by oil, those strains that were not able to grow in the presence of another, were removed from the trial and we selected several ones to formulate a microbial consortium, as an important proposal in water remediation. In these consortia, we work with several strains, as Pseudomonas spp, *Burkholderia* spp, and *Providencia* spp, considering some fungi as *Acremonium* sp. and two yeast strains with the ability of use aliphatic and aromatic hydrocarbons as a carbon source. The ratio of each member into the consortia was also determined. With these data, we are going to be able to initiate trails in soil and water polluted with oil derivatives and estimate the growth condition for a better degradation of the contaminants, and also describe some of the catabolic pathway for aromatic hydrocarbons, using degenerate primers for the naphthalene catabolic pathway, as a proposal to detect strains with a potential to remove organic permanent compounds.

# Isolation and identification of triterpenoid acids with antitumoral effect in a submerged culture of *Humphreya coffeata*

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The fungi are known for being able to produce different compounds with a wide spectrum of pharmacological activities. Among these microorganisms, basidiomycota have been reported to have different metabolites with potencial for antitumoral drugs with less side effects than the current medications available in the market. An example of these are the secondary metabolites called triterpenoid acids found in a well studied basidiomycota *Ganoderma lucidum*.

*Humphreya coffeata* is another basidiomycota, known in Colombia for its medicinal properties, whose compounds with pharmacological activities are unknown. A previous study from our group was the first in the literature to adapt this fungus to laboratory growth conditions and described the potential of this microorganism to be used in cancer therapy. We observed a specific antitumoral activity for leukemia cell lines when exposed to low concentrations of the *H. coffeata* supernatant extract (2500 µg/mL; 80% citotoxicity), while a non-cancerous cell line remained unaffected by it, when exposed at the same concentration and only displayed a significant citotoxic effect (>80%) when exposed to ten times more concentrated extract.

These results led to question which of the compounds present in the culture where responsible for the antitumoral activity. Exopolysaccharide production and bioactivy were previously evaluated, while the bioactivity of the possible triterpenoid compounds present in *H. coffeata* remained unknown.

The present work is focused on evaluating the production of triterpenoid compounds by *H. coffeata* under different submerged culture conditions (two carbon sources: lactose and glucose; two flask geometries: conventional and baffled) at 150 rpm and 30ºC for 20 days, and the characterization of each triterpenoid compound found in each growth condition, and its bioactivity evaluation in different leukemia cell lines.

The results of this study so far have identified that the best *H. coffeata* growth condition to produce triterpenoid acids in submerged cultures is to use glucose as carbon source and conventional flasks (167.1 mg/L in supernatant), Here the oxygen limitation provides a bigger production of secondary metabolites. In a TLC essay it has been observed that up to three triterpenoid acids were produced. This different compounds still need to be identified and evaluated for their citotoxic possible effect.

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# Characterization of putative antiterminator gene e46 of bacteriophage mEp021

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mEp021 coliphage was isolated from human feces and it belongs to a new group of phages non-related to lambdoid phage group. It cannot be induced by UV light and grows mainly at 37°C. However, both groups require of Nus factors from the host cell to develop. It has been reported, that lambda phage employs these host factors to achieve the antitermination process, which consists in supressing the effect of transcription terminators. Hence, it is suggested that mEp021 phage has an antitermination mechanism. *In silico* analysis of mEp021 genome shows a putative antiterminator, called e46, which has a conserved cysteine-rich domain similiar to lambda Q antiterminator. Both share a similar 3D structure , so it is likely that e46 may have an equivalent function. To analyze its function, firstly the e46 gene was cloned in the expression vector pKQV4, inducible by IPTG. Then, we measured its effect in the bacteria growth, showing a low toxicity in its viability. In addition, expression of e46 (26.4 kDa) was confirmed by a SDS-PAGE. This construction has also been tested in different mutant Nus, looking for some complementation. However, we did not observed any effect of e46 in all the mutants, concluding that Nus factors could be acting in another way different to λN complex, but not directly with e46. On the other hand, e46 shows an effect in lambda development, increasing the viral particles, only in nusE71 and nusB5 mutant. It suggested that e46 could act as Q antiterminator, supplying its activity.

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# HilD and PhoP regulate the expression of a novel gene required for *Salmonella enterica* serovar Typhimurium invasion of host cells

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The acquisition of DNA fragments by horizontal transfer events has played a major role in the evolution of pathogenic bacteria. The acquired DNA may encode different factors that confer the ability to survive and replicate in distinct biological niches within an animal or human host causing a bacterial infection and disease. *Salmonella enterica* serotype Typhimurium (*S*. Typhimurium) can produce intestinal or systemic infections in human and animals. Most of the virulence genes of *Salmonella* are clustered in genomic regions denominated *Salmonella* pathogenicity islands (SPIs). The genes SPI-1 genes mediate *Salmonella* invasion of host cells, and thus intestinal colonization leading to enteritis, whereas the genes located in SPI-2 are mainly required for *Salmonella* survival and replication inside host cells, and hence for the systemic disease. Different regulatory factors are involved in the expression of the *Salmonella* virulence genes *in vivo*, as well as under *in vitro* growth conditions that somehow mimic environmental cues encounter by this bacterium in a host during an infection. When *Salmonella* is grown in nutrient-rich media, such as the Luria-Bertani (LB) medium, HilD, an AraC-like transcriptional regulator encoded in SPI-1, induces the expression of the genes within this island; whereas, when *Salmonella* is grown in minimal media, the global two-component system PhoP/Q is required for the expression of the SPI-2 genes. HilD and PhoP/Q also regulate the expression of several other virulence genes located outside SPI-1 and SPI-2. Recently, we reported that the response regulator PhoP induces the expression of the *ecgA* virulence gene located in a *S*. Typhimurium genomic island. Additionally, we have found that the *SL1344_1872* hypothetical gene, located upstream of *ecgA,* is co-expressed with the SPI-1 genes. Here, we show that HilD induces the expression of *SL1344_1872* when *S*. Typhimurium is grown in LB medium; therefore, it was named as *grhA*, 'gene regulated by HilD'. Interestingly, PhoP also positively regulates the expression of *grhA* when *S*. Typhimurium is grown in LB or N-minimal medium, independently of HilD. Moreover, we demonstrate that the *grhA* gene mediates invasion of *S*. Typhimurium into HeLa epithelial cells, RAW264.7 mouse macrophages and NRK-49F rat fibroblasts. Thus, our results reveal a novel factor required for the *Salmonella* invasion of host cells, whose expression is controlled by both HilD and PhoP regulators.

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# Pathogenicity genes and phylogenetic group in uropathogenic Escherichia coli strains

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Key words: Escherichia coli, phylogenetic group, virulence genes.

**Introduction:** *Escherichia coli* strains are divided into 4 groups: A, B1, B2 and D[1]; the strains that cause intestinal infections derive mainly from B2 group and in smaller proportion of group D, while groups A and B1 are categorized as commensal groups[2]. *E. coli* virulence factors are encoded mainly in pathogenicity islands (PAI) of different lengths, giving it the ability to cause urinary tract infections such as urethritis, cystitis and pyelonephritis[3]. **Objective:** To identify the phylogenetic group and virulence genes of Escherichia coli strains in patients from Hospital Juárez de México. **Material and Methods:** DNA extraction of 100 bacterial cultures in patient samples from Hospital Juárez de México was performed following the instructions of the QIAamp® commercial PCR kit and afterwards a PCR was done to identify the phylogenetic group, followed by a second PCR for the detection of 6 virulence genes (fimH, tratT, sfa/focDE, papC, iutA and cnf1). All amplicons were visualized in agarose gels by 2%. **Results:** 65% of the analyzed samples belonged to the B2 group; 25% to D group, and the remaining 10% belonged to groups A and B1. So far, we have found all the genes proposed in this work. **Conclusions:** The phylogenetic group B2 is dominant in the population studied, and although we only have preliminary results in the virulence genes, they correlate with those reported in the literature.

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# Microbial volatile organic compounds (mVOCs) that promote growth in *Arabidopsis thaliana*

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**Background:** While the contribution of diffusible soluble secondary metabolites in bacterial ability to communicate, compete or cooperate with neighboring microorganisms has been actively investigated, volatile organic compounds produced by microorganisms are far less understood[1]. Volatiles are typically small compounds (up to C20) with low molecular mass (100–500 Daltons), high vapour pressure, low boiling point and a lipophilic moiety[2]. Nowadays, a total of 1,093 microbial volatiles have been identified and grouped into several chemical classes[3]. However, biological functions of most microbial volatiles identified to date remain elusive. They are assumed to serve as 1) infochemicals for inter- and intra- organism communication, 2) cell-to-cell communication signals, 3) growth-promoting or 4) inhibiting agents.
**Main goals:** Current challenges in mVOCs research are the identification of volatiles diversity and their role in plant-microbe chemical communication, therefore the goals of this work were: 1) To identify *Arabidopsis thaliana* growth-promoting microorganisms via mVOCs. 2) To analyze the composition of mVOCs and 3) To identify bioactive mVOCs.
**Experimental strategy:** A collection of 40 bacterial strains and one yeast isolated from the endosphere, rhizosphere and phyllosphere of *Agave tequilana*, *Agave salmiana*, *Opuntia robusta* and *Myrtillocactus geometrizans*[4,5], were investigated for the production of mVOCs in the interaction with *Arabidopsis* plants.
In accordance with the goals, four important stages were planned: 1) Screening: Divided Petri plates system were used to evaluate the rol of microbial VOCs on plant growth, 2) Identification: Plant growth-promoting strains via VOCs were selected for the identification of mVOCs using Solid Phase Microextration and GC-MS. 3) Evaluation: mVOCs identified had been used for dose-response experiments.
**Results**: Based on mVOCs effects on *A. Thaliana* plants, four diferent functional groups had been identified in response to mVOCs in *Arabidopsis thaliana* plants: 1) Neutral (no response to mVOCs), 2) Negative (growth inhibition), 3) Slightly positive (primary root growth-promotion), 4) Positive (primary and lateral roots lenght growth-promotion). Around 14 differents compounds had been identified and selected for further studies.

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# Isolation, identification and characterization of nitrogen-fixing bacteria from the rhizosphere of plants inhabiting the mine "El Bote" from Zacatecas, Zac. Mexico.

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Mining site's soils lack organic matter and essential elements such as nitrogen; however, in these kinds of soils, there might be microorganisms with the biological capacity of nitrogen atmospheric fixation which can be help to thrust vegetal life and ecosystem health. An analysis of the rhizosphere of plants inhabiting mine tailings from the mine "El Bote" located in Zacatecas Zac. Mexico was carried out, focusing on the search for rhizobacteria with the biological capacity of nitrogen fixation so in that way we can get to understand the nitrogen biogeochemical cycle's flux and the diversity of these microorganisms from this site. Enrichment cultures in nitrogen-free medium inoculated with soil rhizosphere coming from this mine tailing were made in order to isolate possible diazotrophic bacteria. Once we obtained the strains, a molecular analysis was carried out on the same by first isolating genomic DNA using two alternative protocols specifically for Gram-positive and Gram-negative and then amplifying through PCR the ribosomal gene 16S (rDNA 16S) so in that way through the analysis of nucleotide sequences such strains could be identified. We concluded with the identification of the gen *nifH* through the amplification of this gene using 4 sets of primers specific for diazotrophs species previously reported and 3 pairs of primers degenareted to cover more diversity of nitrogenase genes reported in the diazotroph organisms. We obtained a total of 11 strains that were capable of growing in nitrogen-free medium and the the gene sequence analysis showed that most of them corresponded to nitrogen fixing bacteria genera highly studied like *Rhizobium* and other diazotroph genera also reported like *Serratia*, *Acinetobacter*, *Xanthomonas* and *Streptomyces*. As for the identification of the gen *nifH*, from the 7 pairs of primers tested, only one of the degenarated primers amplified an expected DNA fragment size in all of the strains tested, nevertheless, the sequenciation of one those amplicons showed that it does not match the *nifH* gene.
The results obtained in this work suggest that a considerable diversity of nitrogen fixing rhizobacteria might exist in the rhizosphere of plants that inhabit the mine "El Bote". Molecular methods for detecting functional genes like *nifH* represent a good tool for the identification of nitrogen-fixing bacteria despite of the results; however, an analysis of the great variety of primers that target this gene should be done first in order to get more accurate results.

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# Phenolic compounds and antimicrobial activity of aqueous extracts of *Lippia alba* and *Lippia dulcis* of Cunduacán, Tabasco.

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## Abstract.

*Lippia alba* and *Lippia dulcis* species are widely distributed in the state of Tabasco, its main uses in traditional medicine are in the form of decoction and tea to relieve stomach, respiratory and gynecological disorders. Some therapeutic properties are related to the content of phenolic compounds. This study focused on the study of aqueous extracts of *Lippia alba* and *Lippia dulcis* from Cunduacán, Tabasco, specifically in the content of phenolic compounds and determination of antimicrobial susceptibility. The colorimetric method of Folin-Ciocalteu was used, with dilutions 20 and 30% of *Lippia alba* and *Lippia dulcis* respectively for the determination of phenolic compounds. A standard curve of gallic acid 0.01% was employed as standard and read on a spectrophotometer UV-1800 model RayLabel® 745 nm. For antimicrobial activity by disc diffusion method, *Bacillus cereus*, *Staphylococc*us *aureus*, *Escherichia coli* and *Salmonella spp* were used; in solid medium brain-heart infusion, whom I put them filter paper discs impregnated with aqueous extract, Amikacina® as a positive control and water as a negative control. Concentrations of 1 and 2 µg of samples were used. The results concerning the content of phenols, show that *Lippia alb*a has a higher content of total phenols *Lippia dulcis*, 133.42 mg/g and 44.77 mg/g respectively. With regard to the effectiveness antimicrobial activity it was observed against all strains of the concentration of 2µg with the extract of *Lippia alba*. *Lippia dulcis* while, at the same concentration, present only activity against *Salmonella spp* and *Bacillus cereus*.

**Key words**: *Total phenols, Antimicrobial activity, Lippia alba.*

# Detection of Influenza A virus canine in the metropolitan area of Monterrey, Nuevo Leon.

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**Introduction:** Canine influenza virus is a highly contagious disease belonging to the genus *Influenzavirus A*. Various subtypes of this virus have been responsible of outbreaks in dogs around the world. The matrix gene and their M1 protein are widely used for molecular detection in different species due to this protein is highly conserved. Phylogenetic analyzes reveal 97-98% of identity in nucleotide sequence between human and canine virus. The aim of this study is to determine the presence of *Influenza A virus* in stray dogs through the matrix gene of RNA viral. **Methodology:** Samples were collected from March to December 2015 in two antirabic center of the metropolitan area of Monterrey. A total of 123 sera were analyzed by ELISA HADAS system. In addition 63 nasal swabs and five lung biopsies were tested by RT-PCR. RNA from nasal swabs and biopsies was extracted by TRIZOL reagent. Then, a fragment of 244 bp of the matrix gene M1 was amplified using primers recommended by WHO for typing of *Influenzavirus A*. **Results:** The seroprevalence of anti-M1 antibodies was 100% in canine serum. In addition, an amplicon of 244 bp of the matrix gene of *Influenzavirus A* in samples of nasal fluid 20 (29.41%) and 5 (100%) lung biopsies were amplified. **Conclusion:** These results showed the presence of influenza A virus in stray dogs in the metropolitan area of Monterrey and suggest the importance of detection of the virus in domestic animal species, as well as the susceptibility of humans to these viruses.

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# Infection with influenza A virus H1N1 (2009) of endothelial cells HMEC-1 and its effect on Protease Activated Receptors (PAR-1).

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Introduction

Influenza A virus H1N1 (2009) is considered a global health emergency; it has been described that during 2009 caused between 151.700 and 575.400 deaths worldwide, affecting mostly people under 65 years. Influenza viruses commonly infect the upper respiratory tract resulting in a limited infection, but in severe cases the viruses can reach the lower respiratory tract and cause pneumonia. In this type of infections a severe damage is generated in the pulmonary microvasculature, causing edema, hypoxemia and respiratory failure. Also in patients with severe influenza, it has been described an increase in proinflmatory cytokines denominated “cytokine storm” causing fatal cases, and multiorgan failure. In these inflammatory processes protease activated receptors (PARs), play a determinant role because its activity is directly involved in cytoprotection, regulation of the coagulation cascade and inflammatory processes. PAR-1 is a receptor present in the microvasculature endothelium and its participation in the activation of inflammatory genes through MAPK kinase (ERK 1/2, p38, JNK) is decisive for the translocation of NF-κB to the nucleus, which regulates the expression of such genes. Currently there is not clear information on the participation of PAR-1 during infection of the vascular endothelium with influenza A virus, so in this paper the following is intended.

Objective

The purpose of this study is to evaluate the effect of infection with influenza A virus H1N1-pdm2009 (IAH1N1pdm09) on the expression of PAR-1 and the translocation of NF-κB to the nucleus in the endothelial cells line HMEC-1

Material and Methods

Kinetics of infection was performed with IAH1N1pdm09 at a multiplicity of infection (M.O.I) of one. Total cell proteins, or the cytoplasmic and nuclear fractions were obtained at 15 minutes, 1, 6 and 24 hours post infection, TNF–α was used as a positive control. Later on, Western blot assays (Wb), and electrophoretic mobility shift assays (EMSA) were performed to assess: PAR-1, ERK-1/2, p-ERK-1/2, and IkBα protein amount, or p65 nuclear location.

Results

Infection with IAH1N1pdm09 showed a non-significant increase in the levels of PAR-1 at 6 hours post-infection, the increase is only a fraction of the baseline, but in contrast it demonstrated an almost complete decline when cells were stimulated with TNF-α. The virus stimulated the ERK phosphorylation as noticed at 1h and maintained at 6h post infection at levels comparable to the effect of TNF-α. However, the infection with a M.O.I of one is not a sufficient stimulus to promote the IkBα degradation in the cytoplasm or the p65 translocation to the nucleus in the analysis by Wb, although the effect was already observed after stimulation with TNF-α as expected. Retardation assays corroborated the lack of activation of NF-κB system in the virus infection. These results suggest that the signaling pathways that are activated after the infection of the endothelium with IAH1N1pdm09 do not induce directly the syntheses of proinflammatory cytokines, which depend on the activity of NF-κB, indicating that in the proinflammatory effect of influenza viruses may contribute other routes, one of which could be the coagulation cascade.

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# Isolation of electrochemically active bacteria from a microbial fuel cell

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Electricity generation using microorganisms in a microbial fuel cell (MFC) is a topic of current interest, this technology offers a promising solution to meet the growing energy needs and wastewater treatment, but not all microorganisms have the ability to transfer electrons from the respiratory chain to the electrode, this process is crucial to the operation of an MFC, which are being investigated microorganisms and their interactions with the electrode. The focus of the work is about the isolation and identification of microorganisms in microbial fuel cells, to explore the possible mechanisms involved in the generation of electricity.

The isolation was performed using serial dilutions and plating with different culture media such as nutrient, LB and a synthetic medium was supplemented with ferric ammonium citrate obtain five strains in total. The isolates were tested for their ability to reduce ferric ion (Fe3 +), using the methodology established by Szöllősi et al. (2015) because there is a direct correlation between the reduction of Fe and electrical power generation, this experiment showed three isolates with higher performance in the reduction of ferric ion.

It began to make preparations for a phylogenetic analysis of 16S rDNA to reveal what is most likely gender belonging isolates interest, beginning with the extraction of gDNA and amplification of 16S rDNA using the pair of universal primers 27F and 1492R, with the products obtained are ligated and cloned for further sequencing, and data obtained phylogenetic analysis performed.

The last stage of the project will consist of an electrochemical evaluation of separate interest in MFC, applying a chronoamperometría, to meet the current density generated by microorganisms and cyclic voltammetry to explore the sites of oxidation-reduction, with these data it may infer a possible mechanism involved in electron transport electrode microorganism.

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# Use of the MALDI-TOFMS Biotyper system for rapid identification of microbial strains isolated from pesticide-contaminated soil in Salamanca, Gto.

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Identification of the microorganisms by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) has recently emerged as a rapid and sensitive technology that provides mass spectra of highly abundant proteins (ribosomal). These spectra are unique to individual organism-types and contain *m/z* signals specific to genera, species or sub-species level. MALDI equipment with Biotyper software and suitable databases are widely used in clinical diagnostics, have recently been approved by the FDA and have partly replaced the conventional microbiological techniques such as biochemical identification. The new MS-based technology requires minimal sample preparation, offers short time of spectra acquisition and data analysis and is cost-effective; however, in the analysis of environmental samples additional laboratory tests may be required. The goal of this work was to explore the feasibility of MALDI-TOF-MS Biotyper system for identification of the cultivable microorganisms isolated from contaminated soil in the industrial zone of Salamanca, Gto. (old Fertimex unit). The microorganisms were isolated by two approaches, in order to: (i) find all cultivable species (using a growth medium rich in nutrients) and (ii) to obtain those organisms that are able to resist/metabolize persistent organic pollutants (POPs) such as DDT or its metabolites DDE, DDD (using defined media). The isolated microbial strains were transferred directly from the intact colony on the MALDI target or prepared from protein extraction (ethanol/formic acid) and finally, α-cyano-4-hydroxycinnamic acid (HCCA) in a mixture of 50% MeCN, 47.5% water and 2.5% TFA was added. Straightway, the loaded samples were air dried at room temperature before insertion into the MALDI-TOF (AutoFlex, Bruker) mass spectrometer. Microorganisms identification was based on the comparison of the mass spectrum of the loaded sample with those of reference strains, with the aid of Biotyper software from Bruker and applying standard interpretative criteria. In particular, the Biotyper software compares the mass spectrum of each sample with the reference mass spectra in the database and calculates an arbitrary unit $\log_{10}$ score value between 0 and 3 reflecting the similarity between sample and reference spectrum. In this work, the score values of ≥2.0 were accepted for species assignment, scores of ≥1.7 but <2.0 were considered sufficient for the identification at the genus level whereas scores below 1.7 were considered unreliable. In addition to score values, Biotyper provides the sample match result in a consistency category: A (species), B (genus) and C (no consistency). The overall identification rate was higher than 95%; as the dominants isolated and identified were in their majority strains of the genera, for example, *Pseudomonas, Bacillus, Serratia, Aeromonas, Enterobacter and Lecrercia.*

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# Functional characterization of GrlR, a LEE-encoded negative regulator of enteropathogenic *Escherichia coli*

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EPEC belongs to a group of pathogens that form ‘‘attaching and effacing’’ (A/E) lesions on intestinal epithelia. The A/E lesion is characterized by the localized destruction of the enterocyte apical microvilli and cytoskeleton rearrangements beneath the adherent bacteria, leading to the formation of actin-rich pedestals and intimate bacterium-host cell interactions. The genes required for the formation of the A/E lesion are located within a pathogenicity island known as the locus of enterocyte effacement (LEE), which expression is repressed by H-NS. Ler (LEE encoded regulator) activates the expression of the LEE by disrupting H-NS repression. GrlA is required for the activation of *ler*, whereas GrlR represses the expression of LEE operons. Protein-protein interaction experiments and crystallography showed that the GrlR dimer interacts with GrlA, so it was proposed that GrlR inhibits GrlA activity and therefore the expression of LEE genes.

In the absence of GrlR, the transcriptional activity of LEE genes increases under repressing growth conditions, while GrlR overexpression leads to repression of LEE genes in wild type EPEC and even in EPEC Δ*hns* or *E. coli* K12 Δ*hns*. In this work, we generated GrlR mutants in residues forming the crystal structure interphases of the GrlR-GrlA heterotrimer to investigate their role in GrlR-GrlA heterotrimerization and repression of LEE gene expression using native gels, pulldown assays, western blot, secretion assays and transcriptional fusions. Interestingly, we have identified mutants that no longer interact with GrlA nor repress LEE gene expression, and one mutant is not able to form homodimer complex. Overall, our data illustrates that GrlR may repress LEE gene expression as homodimer and through the interaction with GrlA.

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# Incidence of *Demódex folliculorum* infestation in students from the Universidad Juárez Autónoma de Tabasco, campus Chontalpa.

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## Summary

Demodex infestation (Df) inhabits the hair follicles and sebaceous glands of the face. It has been established that poor facial hygiene, use of creams and makeup, among others, generates its proliferation. This mite has been considered as a cause of skin diseases such as rosacea, acne and demodecidosis. The aim of this study is to determine the incidence and infestation of Df in the university population of Chontalpa campus. An observational, prospective, descriptive study for the selection of patients was performed. Superficial skin biopsies were performed face by the temporary adhesion of a slide with cyanoacrylate in the middle of cheek. Later it was observed under light microscopy to calculate the rate of infestation. In conclusion, Df plays an important role in the pathophysiology of certain skin diseases.

**Key words**: Demodecidosis, *Demodex folliculorum*, Acne.

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# Microbiological analysis of water intended for human consumption in the municipality of Centro Tabasco.

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**Sumary**

The present study was carried out a physiochemical, organoleptic and microbiological analysis according to the NOM-201-SSA1-2002 and NOM-proj-NMX-AA-042/1-SCFI 2008, respectively, in 50 samples of water jug 20 L bulk sale, intended for human consumption, from purifying stuffer located in the south seagull's colony in the town center of the city of Villahermosa, Tabasco. First instance the conditions of purification; sanitation and handling of filling jugs, intended for sale and distribution in these small establishments were analyzed. The results denoted that 52% of the samples present hardness values, alkalinity and residual chlorine above the permissible limits (210, 87 and 0.2 ppm respectively). Microbiological analysis showed that 84% of the samples denoted acceptable values of total coliforms (<1.1 NMP / 100 mL). The count of mesophilic aerobic bacteria, said that 76% of oscillated samples below 100 UFC / 100 mL, complying with proposed for this indicator limit, while the remaining 24% comprised ranges between 500 and 999 UFC / ml, exceeding the permissible limits. Pseudomonas in the presence of 5% of the samples was identified, indicating poor purification process and lack of hygiene in washing and filling carboys. Concluding that the purified water sold in bulk is high water hardness, with poor hygienic quality, so it is recommended that health authorities of the state increase the levels of control in such establishments, which have become public health risk.

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# Absence of alcohol dehydrogenase (ADH1) activity in *Mucor circinelloides* increases the mouse tissue invasivity of the fungus

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*Mucor circinelloides* is a dimorphic fungus that is considered as an opportunistic pathogen, whose metabolism and morphogenetic pattern are influenced by oxygen tension and the carbon source in the culture medium.

It has been described that in *M. circinelloides* the *adh1* gene, which encodes for an alcohol dehydrogenase (Adh1), is involved in the last step of the synthesis of ethanol, but has also been associated with yeast-growth in anaerobiosis.

In the dimorphic pathogenic yeast *Candida albicans*, the morphogenetic changes and biofilm formation are important for pathogenicity; these changes are modulated in part by ADH activity. Is in our interest to determine whether the product of *M. circinelloides adh1* gene contributes to the virulence throughout the mice tissue invasion. In this work, we compared the level of virulence of an *adh1*$^{-}$ mutant strain (M5), the wild-type strain (R7B, *adh1*$^{+}$) and an M5-derived transformant containing the wild type *adh1*$^{+}$ allele (M5/pEUKA-*adh1*$^{+}$), using as a biological model non-immunocompromised mice.

Our results indicated a higher lethality when mice were infected with the mutant strain M5, generating 60% mortality, meanwhile the strains R7B and M5/pEUKA-*adh1*$^{+}$ were totally avirulent in the same lapse of time. In addition, we performed histological analysis in different organs where mucormycosis has been associated, such as brain, lungs, spleen, liver, and intestine, to determine the fungal morphology associated to each tissue tested. Moreover, the fungal load in these tissues was estimated by real-time PCR; our results showed that, in general, the mutant strain showed a greater accumulation in all the tissues analysed, as compared to wild type.

These observations strongly suggest that in *M. circinelloides* the alteration of ethanol metabolism contributes to the virulence of the fungus. Based on the results described, we intend to advance in the elucidation of the molecular mechanism by which such metabolic alteration contributes to the virulence of *M. circinelloides.*

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# Toxin genes PirA and PirB type in *Vibrio parahaemolyticus* strains isolated from shrimp.

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The "Acute Hepatopancreatic Necrosis Disease" (AHPND) or "Early Mortality Syndrome" (EMS) is a disease of shrimp species *Penaeus monodon* and *Litopenaeus vannamei*. In 2013 Nayarit was the first state where AHPND began in Mexico.The production fell to 67%, compared to production shrimp in 2012.

In 2015 the pVA1 and pVPA3-1 plasmids were identified in strains *of V. parahaemolyticus* causing AHPND. Both plasmids have similar characteristics: a GC content of 45.9%, a size of 70 and 69 Kbs respectively, pVA1 has 90 open reading frames and pVPA3-1 has 92 ORFs encode transposase and proteins used versus insect (Pir toxins), which are encoded by genes type PirA and PirB, these located within a 3.5 Kb fragment flanked with inverted repeats of a sequence encoding transposase.

The PirA and PirB type genes with a size of 336 bp and 1317 bp , encode proteins of 13 and 50 kDa respectively. These toxins are responsible for the AHPND.

Through nested PCR with primers AP4 (AP4F1: 5'ATG AGT AAC AAT AAA ATA GAA CAT AC3 '; AP4R1: 5'ACG ATT TCG ACG TTC CCC AA3' and AP4F2: 5'TTG AGA GTG ATA CGG GAC GG3 '; AP4R2 : AGT CAT 5'GTT AGC GTG ACC TTC3 ') in this work we identified genes and toxins PirA and PirB type in 12 strains of *Vibrio parahaemolyticus* isolated in Nayarit in 2006 and 2007 plus we identified the strains with rep -PCR using palindrome sequence as a primers and corroborating by $GTG_5$ PCR amplification of the region encoding the 16S rRNA.

Two strains from the 12 strains analyzed produced amplicons by PCR using primers AP4, which indicates that there have been strains of *V. parahaemolyticus* with genes of the toxins PirA and PirB type since 2006 in Nayarit without causing AHPND, which probably indicates that besides to these genes are required other factors detonates the disease.

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# The HPV16 E6 oncoprotein variants affects the expression of cadherins in cell C33-A cells

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**INTRODUCTION:** Cervical Cancer (CC) is the fourth leading cause of death by neoplasias in women and Human Papillomavirus 16 (HPV16) is in more than 50% of these CC, becoming the most common high risk virus worldwide. With this the main risk factor for CC is the persistent infection with high risk Human Papillomavirus (HPV-HR). It has been described many genetic variants of HPV 16. These variants are classified into four major lineages and 9 sublineages, according to its original geographical distribution. Epidemiological and experimental evidence suggests that HPV16 variants have different oncogenic potential. Previously, our research group reported that HPV16 E6 oncoprotein variants transfected into C33-A cells can differentially deregulate the genes expression related to cancer. Cadherins are important proteins in cell adhesion and their aberrant expression has been reported in various types of carcinomas.

**OBJECTIVE:** To evaluate the effect of the HPV16 E6 oncoprotein of AA-a, AA-c, E-G350, E-C188/G350 and E-A176/G350 variants in the expression of E-cadherin, N-cadherin, K-cadherin and Cadherin-9 in C33-A cells compared to the E-Prototype (EP).

**MATERIAL AND METHODS**: C33-A cells were stably transfected with the HPV16 E6 oncoprotein of the variants AA-a, AA-c, E-G350, E-C188/G350 and E-A176/G350. The expression of E-cadherin, N-cadherin, cadherin and cadherin-K-9 was determined by confocal fluorescence microscopy.

**RESULTS:** The HPV16 E6 oncoprotein variants AA-c, E-G350, E-C188/G350 and E-A176/G350 differentially affects cadherin expression in C33-A cells. Interestingly, we found that E-cadherin expression decreased in cells C33-A cells transfected with HPV16 E6 while N-cadherin and K-cadherin is overexpressed in C-33A/E-G350 and C-33A/E-P respectively.

**CONCLUSION:** The HPV16 E6 oncoprotein variants differentially affects E-cadherin, N-cadherin and K-cadherin expression compared with E-Prototype in C33-A cells, which could give them different migration potential.

**Keywords**: HPV16, Cadherins, E6 oncoprotein variants, C33-A cells.

# Dissecting microbiome functions in cacti: lessons from seed-transmitted endophytes

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Microbial symbionts account for survival, development, fitness and evolution of most eukaryotic hosts. These microorganisms together with their host form a biological unit known as holobiont. Recently, our studies had revealed that the cacti holobiont comprises a diverse and abundant microbiome, which might be important for its adaptation and survival in arid ecosystems. To dissect the functional capabilities of the cacti microbiome, we focus on seed-borne bacterial endophytes that are vertically transmitted through seeds and might contribute to cacti seedlings' fitness. Our strategy include culture-dependent and independent techniques to evaluate the composition of seed-transmitted bacterial endophytes in cacti of the subfamilies *Opuntioideae* and *Catoide*ae. Then, we make use of genomic data from isolated bacteria to identify enriched functions that correlate with plant growth promotion and stress tolerance. *Opuntia robusta* seedlings and *Arabidopsis thaliana* will be used to assess the impact of seed-borne strains on plant fitness under drought stress. Finally, we attempt to reduce the seed-borne endophytic community in *O. robusta* seedlings by employing antibiotics in order to correlate the loss of certain taxa with plant performance under drought. Our results to date show that cultivable cacti seed-borne bacteria are represented by members of *Bacillus*, *Paenibacillus, Psychrobacillus*, *Agrococcus*, *Nocardiopsis*, *Staphylococcus* and *Leclercia,* being *Bacilli* the most abundant taxa. *Bacillus, Staphylococcus* and *Leclercia* isolates match with abundant OTUs from endosphere and rhizosphere of adult cacti, while the others were present in low abundance. *Agrococcus*, *Staphylococcus* and *Psychrobacillus* genomes possess traits related to growth promotion and survival to drought such as trehalose and butanediol biosynthesis, ACC deaminase and ROS degradative enzymes. Even though antibiotic treatment inhibited rooting of cacti seedlings in a concentration dependent manner, a mix of tetracycline, rifampicin and carbenicilin inhibited *Staphylococcus* on cacti seedlings and *in vitro*. This work will help elucidate the functional and ecological role of seed-borne bacteria during the first stages of cacti development.

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# Isolation and identification of seed-associated bacteria from Guamuchil (*Pithecellobium dulce* (Roxb) Benth.)

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## Abstract

Microorganisms – both bacteria and fungi – that associate with plants can have beneficial, neutral or harmful effects on the host. Well-known examples of beneficial plant-microbe interactions include symbioses such as nitrogen fixation by root associated Rhizobiales, however multiples examples of antagonic roles by potential plant pathogens are also reported. Unique bacterial populations are typical for different plant organs such as leaves, roots and seeds, and can be found on the surface or within the plant tissue. Microorganisms which colonize inner tissues without causing any symptoms of disease are defined as endophytes, and they may play an important role in the plant development i. e. providing anti-fungal activity or as growth promoting bacteria.

Guamuchil (*Pithecellobium dulce* (Roxb) Benth.) tree, a plant belonging to the Fabaceae family, is an occurring component of the Mexican deciduous and subdeciduous forests. It is considered as a multipurpouse tree, is used as live fence in cultivated land, and its fruit – a bent and round shape- is used as food for cattle and humans. The seeds are protected by a white, pink or a reddish pulp, which is the edible part. Bacterial endophytes have been isolated from seeds of leguminosae species such as *Phaseolus* species, however microorganisms endophytes of trees are poor documented.

This study is intended to isolate and identify seed-associated bacteria from fresh seeds of Guamuchil. By previuos surface sterilization with ethanol, sodium hypochlorite and sterile water in order to eliminate exogenous bacteria from the seeds, and incubated by many days at room temperature in a complete agar medium we have found a variety of bacteria both Gram positive and Gram negative, which are expected to be identified by molecular tools in order to learn more about these endogenous bacteria. The results of this analysis will be presented during the meeting.

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# Antibody generation against the recombinant protein Gp70 from *Sporothrix schenckii sensu stricto* and *Sporothrix brasiliensis*

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Sporotrichosis is the most frequent subcutaneous mycosis in Latin America. Until recently, this disease had been attributed to a single etiological agent, the fungus *Sporothrix schenckii*. A recent phylogenetic study proposed that *S. schenckii* is actually a complex of cryptic species, at least five of which have clinical interest: *S. globosa*, *S. brasiliensis*, *S. lurei*, *S. mexicana* and *S. schenckii sensu stricto*.

Members of the *S. schenckii* species complex are dimorphic fungi. The mycelial morphotype penetrates the human host, through skin abrasions produced by contaminated plants or animals, before undergoing the morphological switch to yeast-like cells. In addition, the zoonotic transmission by domestic cats is of clinical and epidemiological importance.

There are some reports dealing with a cell wall glycoprotein of 70 kDa (Gp70), which has shown to mediate the binding of yeast cells to the dermal matrix and to fibronectin. Gp70 also plays a pivotal role as an antigen, which can modulate the host immune response.

Cell wall antigens and anti-cell wall antibodies may be the basis for developing specific and sensitive serologic tests. The main challenge for developing a suitable diagnostic test is obtaining a species-specific antigen, this becomes an even more complicated goal because of the high cross-reactivity observed for glycoconjugates present either on the cell wall or in culture filtrate preparations.

Here, we aim to develop a serological test for the detection of *S. schenckii* and *S. brasiliensis*, based on an enzyme linked immunosorbent assay, using a recombinant not glycosylated Gp70 as an antigen, in order to avoid cross-reactivity.

The protocol for the induction of the recombinant protein in *Escherichia coli* has been established in our work group. Our progress, so far, consists on the purification of the *S. schenckii* and *S. brasiliensis* recombinant proteins (SsGp70 and SbGp70), the generation of antibodies against SsGp70 and their titration, showing a good response against the antigen. Besides, we have performed a preliminary dot blot, in which we obtained a positive result of the antibodies against the recombinant protein

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# Phenotypic diversity of *B. coahuilensis* and increase of carbon utilization in experimental evolution

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*Bacillus coahuilensis* was isolated from Cuatro Ciénegas in the Chihuahuan Desert (Coahuila, North Central México). Genomic analysis suggested that it possessed adaptations to an oligotrophic environment, particularly to low phosphorus concentrations. A microevolutionary analysis of three *B. coahuilensis* strains revealed genomic differences and also phenotypic variation, particularly in preferences for carbohydrate utilization. The aim of this study was to evaluate the capacity for the evolution of novel traits of one *B. coahuilensis* strain (m2-6) propagated in medium with two different concentration of phosphorus. We did daily transfers for 100 generations in two different environments consisting phosphate defined medium (PDM) with high (HPDM) and low (LPDM) concentrations of phosphorus using glucose as a carbon source. Three biological replicates were inoculated for each condition. We used the Biolog system to determine the possible adjustments in the evolved populations regarding carbon source utilization, as we expected non-directed adjustments to a novel environment to impact different aspects of metabolism. Absorbance and Dual wavelength data (DWD) values were used to calculate the fold changes in the utilization of the carbon sources for the six evolved population in comparison with the ancestral strain. DWD values were also used to quantify the phenotypic diversity using Shannon's index of diversity. The evolved strains, regardless of the P concentration in the media used for transfers, exhibited a general increase in the capability of using different sugars, with a parallelism of the different populations in the ability to use glucose, dextrin, acetic acid, pyruvic acid, maltose, maltotriose and aromatic compounds (thymidine, uridine, adenosine, and inosine) in the evolved populations in comparison to the ancestral strain. Similarly all evolved populations showed a decrease in the use of D-ribose, succinic acid, L-arabinose, D-xylose, mannan, 3-methyl glucose, and turanosa. The Shannon's index indicated differences in the capability of use carbon sources between ancestral strains an evolved population. Also we observed differences in the robustness of use of the different resources between the evolved populations. We conclude that the phenotypic optimization that occurs in any given medium that selects for optimal growth is probably the result of global changes in gene regulation in the evolved populations, that are therefore pleiotropic, and reflect the absence of a substrate-specific adaptation as a key driver of evolution.

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# Recruitment of celular factor RTN3 in the viral replication complex on HMEC-1 cells infected with Dengue virus.

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Introduction: Several studies have defined that replication of Dengue virus genome, it occurs in structures knowing as replication complex which are assembled into new compartments originated from endoplasmic reticulum membranes. The formation of this new architecture depends on viral and cellular factors. In this respect it has been suggested that celullar protein RTN3 play an important role in the process of reorganization of membranes during viruses RNA infections. In this study we determine whether the RTN3 protein is found in areas of viral replication, which might suggest that this factor is being used by infection to form the reorganization of the membranes during replication of dengue virus. The study of cellular factors used in this process is a strategy for the development of new antiviral targets.

Materials and methods: First was evaluated RTN3 expression in cells permissive to infection with dengue virus. Assays of western blot and immunofluorescence was performed. Later, kinetics of infection of 12, 24 and 48 hours in cells infected with DENV -2 were made, and evalueted the co-location of RTN3 and viral NS3 protein by confocal microscopy. The NS4A sequence of DENV-2 was cloned in the pCMV-4-Flag vector and subsequently HMEC-1 cells were transfected with this construct assessed by analysis immunofluorescence at 12 and 24 hours was performed, finally co-localization was analyzed NS4A with RTN3 by confocal microscopy. We determined the percentage of colocalization between the regions of interest at respective time points based on the quantification of colocalization coefficients.

Results. Expression RTN3 cellular factor was identified in the cell line HMEC-1, and was found in a reticular-perinuclear pattern. During infection with DENV-2, RTN3 co-located with the viral NS3 replication marker in perinuclear-cyoplasmatic foci at 12 and 48 hours, being in late stages as co-location having a linear correlation index 0.67 average without stadistical difference. In endothelial cells transfected with the FLAG-NS4A we determinate the linear correlation between NS4A and RTN3 and at 12 and 24 hours was evident, with an average of 0.84 without stadiscical difference. In addition, distribution observed in perinuclear-cytoplasmic foci similar to those appreciated during infection.

Conclusions. The data obtained in this work, showed that the cellular factor RTN3 is present in zones of viral replication. This suggests that could be associated with the NS4A protein involved in the reorganization of membranes dynamics. Besides, the RTN3 protein is recruited and possibly in early infection stages as an element for training or induction of viral replication complexes.

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# Identification of *Rhizobium tropici* CIAT899 Genes Involved in Benzo[a]pyrene Resistance or Degradation

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## Abstract

*Rhizobium tropici* CIAT899 is highly tolerant to environmental stress such as acidity, high temperature and heavy metals. It was recently reported that it is able to tolerate and degrade the carcinogenic and teratogenic compound benzo[a]pyrene (BaP) (González-Paredes *et al*., 2013). The aim of this work was to identify the genes involved in the resistance and/or degradation of BaP. In order to search for candidate genes, a 1000 mutant strain library was obtained with mTn5*gusA1* minitransposon. *R. tropici* CIAT899 was exposed to different concentrations of BaP to evaluate and establish the growth rate, minimum inhibitory concentration and dose-effect. We found that 0-100 µg $mL^{-1}$ concentrations of BaP did not affect CIAT899 growth but inhibited other bacteria. However 250 and 500 µg $mL^{-1}$ of BaP caused a delay in growth for 24 and 48 h, respectively. Growth was re-established at 168 h compared to the treatment without BaP. Two mutants were selected for presenting high tolerance and accelerate growth rate in presence of 500 µg $mL^{-1}$ of BaP compared to the WT. Besides the phenotypic characterization, the genomic and transcriptomic analysis of these mutants is being performed.

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# Effect of psidium guajava on periodontopathogenic bacteria.

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Abstract

The periodontal disease is an inflammatory process occurring in the surrounding tissues of the teeth in response to an accumulation of bacteria (plaque dental), its etiology is polymicrobial, since a large number of bacterial species, mainly anaerobic and microaerófilas bacteria are involved. This work aimed, to determine the effect of antibacterial ethanolic extract and aqueous guava leaf (Psidium guajava) against bacteria in periodontal disease as well as insulation, reproduction and standardization of the bacterial cultures obtained from patients with periodontal lesion. *The antimicrobial activity of guava is attributed to flavonoids, guaijaverin, quercetin and tannins, having effects on a large number of periodontopathogenic bacteria as it is the case of Aggreagatibacter actinomycetemcomitans, Porphyromonas gingivalis,* Intermediate Prevotella, Fusobacterium nucleatum, Staphylococcus Mutans, Staphylococcus Aureus, Staphylococcus Sanguinis, Streptococcus Mitis, Actinomyces, Escherichia coli, Streptococcus Salivarius among others. Based on this review, Psidium guajava could be used as an adjuvant in the treatment of periodontal disease.

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# Expression of IRE1α as a prognostic marker of cervical cancer: an immunohistochemical analysis.

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Despite screening and vaccination schemes, cervical cancer remains the second most common cancer in women over 14 in Mexico. Although it has been recognized that infection with high-risk types of human papillomavirus (HR-HPV) is major risk factor for cervical carcinogenesis, it is not sufficient to induce neoplastic development. Recently it has been reported that the Unfolded Protein Response (UPR) can be modified by HPV16 infection and is altered in cervical cancer. IRE1α (Inositol Requiring Enzyme 1 alpha) is a proximal sensor and initiator of the UPR whose transcript levels were downregulated in human foreskin keratinocytes after the expression of E5 protein of HPV16, suggesting that E5 protein may be able to exert an effect on ER stress induced by expression of viral proteins thus allowing the survival of the infected cell and the establishment of a persistent infection. IRE1α has not been tested in biological samples from the cervix and has not been determined whether there is any relationship between its pattern and level of expression and different precursor stages of cervical cancer or the presence of HPV 16. This work aims to contribute to the search for molecular markers that correlate with neoplastic progression in infected cervical cells by HPV16. To do this, we proposed to evaluate whether there is any relationship between the expression of IRE1α with neoplastic progression of the cervix and the presence of HPV16. Therefore, tissue specimens from normal, preneoplastic y neoplastic cervix were analyzed for expression and localization of IRE1α by immunohistochemistry and also the presence of HPV 16 was determined. IRE1α immunoreactivity was observed primarily in the cytoplasm and its expression pattern and localization within the ectocervical epithelium was different between normal cervix without HPV16 infection and neoplastic and preneoplastic cervix with HPV16 infection. The pattern and level of expression of IRE1α could help identify those patients positive HPV-HR with a recognized risk of progression to cervical cancer. In addition IRE1α could be used as surrogate biochemical marker of the expression E5 and HPV16 integration.

Key Words: Inositol Requiring Enzyme 1 alpha/Endoplasmic Reticulum to Nucleus Signaling 1 (IRE1α/ERN1), Unfolded Protein Response (UPR), Cervical cancer , Human papillomavirus 16 (HPV16).

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# Isolation and analysis of membrane microdomains in *Escherichia coli*.

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A common feature to all living cells is the presence of a lipid membrane that defines the boundary between the inside and the outside of the cell. Proteins that localize to the membrane serve a number of essential functions, for example mediation of signal transduction and protein secretion. In eukaryotic cells these proteins are often localized in membrane microdomains, commonly referred to as ‘‘lipid rafts’’ or ‘‘membrane rafts’’, enriched in certain lipids, such as sterols and sphingolipids. Interestingly, a group of proteins with SPFH domains (Stomatin, Prohibitin, Flotillin and HflC/K), having structural and recruitment functions, have been shown to be associated to the eukaryotic lipid-rafts and are therefore used as lipid raft markers. The Escherichia coli chromosome encodes for four SPFH domain-containing proteins, although their function has not yet been established. Recent results indicate that mutants in these proteins affect the BarA/UvrY signaling pathway. These findings prompted us to suggest that bacterial membranes also contain membrane microdomains. Here, we present the development of a simple method to obtain Detergent Resistant Membranes (DRMs) from *E. coli* inner membranes. The results of a proteomic analysis of these isolated DRMs are also discussed.

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# Production and partial characterization of bacteriocin-like substances from different strains of the *Streptomyces* genus.

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*Streptomyces* is a genus of Gram-positive bacteria able to grow in various environments, with a similar shape as the shown by filamentous fungi (Ōmura et al., 2001); the most interesting and important property of the *Streptomyces* strains is their ability to produce secondary metabolites such as antifungal, antiviral, antitumor and anti-hypertensive compounds and also, they produce a large amount of extracellular enzymes of interest in the industrial area (Demain, 1999; Li and Townsend, 2006). The major production of these compounds occurs by the secondary metabolism, which is a process where not essential products for the producing organism are formed. However, the products often originate ecological advantages and consequently confer greater biological competitiveness, and are mostly expressed in the deceleration phase and the stationary phase (Kirk et al., 2000; García, 2010). Some of these secondary metabolites are antimicrobial peptides (AMP), which are cationic host defense peptides, with a variable number of amino acids in their structure (from five to one hundred), involved in complex mechanisms of action related to the interaction with the pathogen through its membrane, or affecting internal targets (Téllez and Castaño, 2010; Brown and Hancock, 2006). The use of AMP´s represent an alternative with advantages over other compounds in several areas of human interest, for example, it has been proposed the use of some peptides as therapeutic potential in the treatment of burns and ulcers (Murphy et al., 1993), for the control of infectious diseases such as mastitis in the veterinary area (Islas-Rodríguez et al., 2009) or their use as preservatives in food industry (Hernández, 2010). Few studies about the production of AMP's from Streptomyces strains have been developed. Here we present a study of how growth conditions, such as different mediums and temperatures could affect the production of bacteriocin-like substances from different strains of *Streptomyces* genus. Those were tested against several pathogenic bacteria of interest in food industry, and so we demonstrate the proteinaceous nature of these compounds which activity was partially characterized under different conditions of temperature, pH and presence of proteolytic enzymes. It was shown that specific medium YEME (Shepherd et al., 2010) was better to use for bacteriocin-like substance production, even having good results in both temperatures used (28°C and 37°C), while Tryptic soy broth showed to have a greater cell growth but less antimicrobial activity and minimum médium showed few growth and activity. The semi-purified bacteriocins showed good stability at temperature and pH ranges, but loss of activity was detected when proteolytic enzymes treatment were performed.

# Identification and Molecular Detection of *Staphylococcus spp.,* from Uterine Cervix Scrapes

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Cervical cancer (CC) is the third most common cancer in women and the second leading cause of death. In Mexico, CC shows an incidence rate of 15.5% and 12.8% of mortality. This cancer is directly related with the persistent Human Papillomavirus (HPV) infection; however, it has been suggested that HPV infection is necessary but not sufficient for cervical cancer development. In general, the microbiota is known to avoid the invasion of pathogens modulating the pH and other functions. Disturbances in the microbiota, may be involved in a number of diseases, including cancer gastric. In the case of the cervix-vaginal microbiota, it has been observed that there are changes in the community structure of microorganism, showing mainly a decrease of *Lactobacillus spp*.; disturbances in the geni and many other facultative or anaerobic species. Thus, there is the possibility that disturbances in the vaginal environment, will generate more susceptible to growth of pathogenic microorganisms in the cervical area, promoting inflammation and probably contributing with the carcinogenesis. Recently, applying the Next Generation Sequencing analysis in cervical precursor's lesions, *Staphylococcus* spp., was one of the most frequent genus presented in cervical lesions. In order to know the changes of the microbiota presented in the cervical lesions, the goal of this work is to determine the *Staphylococcus* species in the cervical lesions. Cervical scrapes from CC, and women without cervical lesion were analyzed by *tuf*-PCR gene. So far, the present data are showing that *Staphylococcus* genus has a higher concentration in normal cervical epithelium, than in CC, and that the *Staphylococcus epidermidis* specie is more predominant in cervix scrapes. These data could suggest that *Staphylococcus spp*., could play a possible role as co-factor in the cervical carcinogenesis.

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# Phenotypic plasticity of *Bacillus* isolates from Coahuila, Mexico.

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One common phenomenon in organismal biology is phenotypic plasticity: the capacity of a given genotype (or individual) to change its phenotype in response to a change in the environment. Phenotypic plasticity determines tolerance ranges of species when facing environmental challenges. Reaction norms can be applied in evolutionary biology to characterize phenotypic plasticity in a diversity of traits. The component functions of reaction norms can themselves be considered as traits of an individual or genotype. Understanding the causes and consequences of individual or species differences in phenotypic traits is of high importance to evolutionary ecology.

Strains of the genus *Bacillus* were isolated from the Churince, Cuatro Ciénegas, Coahuila, Mexico. These have shown to have different qualitative phenotypes between species at a morphological and physiological level but quantitative phenotypes, as norms of reaction, might also be a species-trait and be explained by evolutionary history. Since the different *Bacillus* species isolated from the Churince pond have shared the same physical-chemical conditions for hundreds of years, we have a chance to ask whether different isolates/species exhibit differences in their phenotypic plasticity to these environmental parameters. In this work we determined the norms of reaction for six strains of *Bacillus subtilis* and six strains of *Bacillus cereus* measuring growth rate and survival to different environmental factors (temperature, concentration of NaCl and UV radiation). Regarding UV-light resistance, thus far, we did not found differences between the two species, as all the strains tested showed similar survival rates. Temperature appears as one of the factor of greatest effect on the ecology and adaptation of organisms, including bacteria. We found that the *subtilis*-species tolerate higher temperatures (growing at 47°C and even 52°C) as compared to the *cereus*-species for which growth rate significantly decreases at temperatures above 47°C. The results showed that for temperature, reaction norms grouped the *Bacillus* species in a similar manner as taxonomic techniques (morphology, physiology and biochemical tests), the phenotypic plasticities showed strong evidence of differential adaptive characteristics associated with evolutionary history. We will discuss this and other data regarding reactions norms for the natural *Bacillus* spp. isolates.

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# Regulatory mechanisms controlling the expression of the *ecp* fimbrial operon in *Citrobacter rodentium*

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*Citrobacter rodentium* is a bacterium that causes colitis and transmissible murine crypt hiperplasia, which shares 67% of its genes with enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC). EPEC is one of the major causes of infantile diarrhea and EHEC causes hemorrhagic colitis and the hemolytic uremic syndrome (HUS), witch could lead to kidney failure and be fatal. *C. rodentium,* EPEC and EHEC produce the A/E (attaching and effacing) lesion on the surface of intestinal epithelial cells, which are mediated by the gene products encoded within a pathogenicity island known as the LEE (Locus of enterocyte effacement). In addition, adherence to different environmental substrates and reservoirs, as well as to host epithelial cells is often mediated by multimeric filamentous structures known as fimbriae or pili. The *E. coli* common pilus is present in commensal and pathogenic *E. coli*, and also found in *C. rodentium*, has been shown to play a role in pathogenic *E. coli* interactions with environmental reservoirs or host epithelial cells.

As for *E. coli,* the *C. rodentium ecp* operon is a cluster of five genes (*ecpABCDE*) encoding proteins involved in the assembly of the fimbria, which expression is favored during stationary phase and in static DMEM cultures at 26°C. However, the *ecpR* gene, encoding the positive regulator of *ecp* in EPEC is not at the start of the operon but downstream in the opposite direction and separated by the *creR* gene that is not present in *E. coli.* Interestingly, while EcpR does not seem to be expressed nor have a role in *ecp* regulation in *C. rodentium,* we found that *creR*, encoding a putative phosphodiesterase, is essential for *ecp* activation.

Using transcriptional fusions to the *cat* reporter gene we have identified a regulatory sequence, named Distal Regulatory Element (DRE), that is essential for *ecp* expression. Moreover, we found that the global regulators IHF and H-NS regulate, as in EPEC and EHEC, *C. rodentium ecp* expression in a positive and negative manner, respectively, and that the presence of CreR was still needed even in absence of H-NS. CreR is not a key regulator for biofilm formation but its overexpression exerts a negative effect. CreR does not act through c-di-GMP binding proteins containing the PilZ domain, as *C. rodentium* mutants in the genes encoding the PilZ-like proteins YcgR and BcsA still expressed *ecp* as the wild type strain. Interestingly, a *creR* deletion mutant is attenuated during infection in mice, but not a *ΔecpA* strain, suggesting that CreR controls the expression of at least one additional gene encoding a virulence determinant. We are currently elucidating the CreR regulatory cascade, which offers the opportunity to reveal new aspects of A/E pathogenicity.

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# Phenotypic differentiation of aquatic *Bacillus* species

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Most the species under the genus *Bacillus* are Gram-positive, spore-forming, motile rods, catalase- and oxidase-positive, and aerobes or facultative anaerobes. However, it has been reported that some alkaliphilic *Bacillus* spp. have particular phenotypic traits, like *Bacillus horti,* that stains as Gram-negative. The aim of this study was to compare the consistency of some phenotypic traits among *Bacillus* spp. isolated from aquatic and non-aquatic environments. We evaluated some phenotypic traits like Gram-stain, catalase, oxidase, and antibiotics' resistance. Our results indicated that most *Bacillus* spp. considered as non-aquatic, like *Bacillus cereus, Bacillus subtilis,* and *Bacillus megaterium* were more consistent with the classical phenotype of the genus. In the particular case of *Bacillus pumilus* some of the isolates stained as Gram-negative. For *Bacillus* spp. considered as aquatic like *Bacillus horikoshi, Bacillus marisflavi, Bacillus aquimaris*, and *Bacillus coahuilensis* phenotypes such as catalase, oxidase production and resistance to some antibiotics indicated that the aquatic *Bacillus* spp. had the expected behavior for the genus. However, these isolates stained as Gram-negative. The Gram-negative phenotype of the aquatic *Bacillus* spp. could be the result of some modification in cell wall thickness or composition, or possibly membrane differences. These phenotypes could be an adaptations to an aquatic life, or ancestral to the terra-*Bacillus*. It will be interesting to investigate if these trait evolved independently in the different *Bacillus* spp. through gene loss of through gene acquisition of by lateral gene transfer.

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# Antiviral and immunomodulatory effect of six poliphenolic compounds in cells infected with dengue virus serotype 2

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Background. Dengue represents one of the main viral diseases transmitted by vectors. There is not a specific antiviral treatment or vaccine fully effective versus this disease; some studies have shown that the immune response is related to the development of severe forms of the disease by different mechanisms. Objective. Evaluate the antiviral and immunomodulatory effect of six polyphenolic compounds in cells infected with dengue virus serotype 2 (DENV-2). Materials and methods. The antiviral effect was determined in C33-A cells by the method of lytic plaques forming units under three treatment regimens (before, during and after infection). In studies of immune response, macrophages U937 and U937-DC-SIGN were infected with DENV-2 (MOI= 1) and the production of the cytokines IL-1, IL-6, IL-10, IL-12 TNF-α was determined by ELISA at different times post-infection to finally determine the effect of compounds on such cytokine synthesis. Results and conclusions. Three of the six studied compounds had antiviral effect in the post-infection treatment and in the preincubation of virus with each compound, whereas the treatment during the infection was not effective. In studies of immune response higher levels of cytokines were obtained in the macrophages U937-DC-SIGN infected compared to macrophages U937 infected. Nowadays we are currently evaluating the effect of the polyphenolics compounds on the cytokine production; and perhaps, together with the observed antiviral effect and future studies, we can suggest some molecule as a posible therapeutic against dengue.

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# Detection of *Pseudomonas aeruginosa* in spinal liquid for Loop-mediated Isothermal Amplification (LAMP).

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*Pseudomonas aeruginosa* is a pathogen gram-negative bacillus opportunistic responsible of 10 - 15% nosocomial infections; principally in infants. *P. aeruginosa* is associated with adverse health consequences to patient´s that can be fatal.
Although conventional microbiological method for identifying *P. aeruginosa* from cinical sample are available; however, this method requires three to four days. The method LAMP (Loop-Mediated Isothermal Amplification) is a technique that allows to obtain results in a short time, with high specificity, sensitivity and economic. This technique is based in the strand displacement of DNA synthesis performed by the Bst DNA polymerase, fixed a temperature. Requires three oligonucleotide sets, which are used to recognize six regions of the target sequence. The objective of this work was to develop and implement the LAMP method for detection of *P. aeruginosa* in sample of spinal liquid (SL) of neonates, obtained from a Children's Hospital for educational purposes. Methodology, for the achievement for the technique of LAMP an analysis was realized in silico of the sequences of *P. aeruginosa* (NCBI), later a multiple alignment was done with the program MUSCLE (www.ebi.ac.uk) to obtain the sequence consensus. The sequences of the specific oligonucleotides were analyzed in the program OligoAnalyzer (www.idtdna.com). Later there was realized the extraction of DNA of the samples of Spinal Liquid by means of commercial kit of mark Quiagen; as positive control was used a bacterial strain of *P. aeruginosa* isolated in Laboratory of Immunology of the UMBA. For the reaction of the LAMP there used betaine, $MgSO_4$, HNB, Bstpol, dNTPs, buffer, oligonucleotides specifics BIP, FIP, F3 and B3, the mixture was incubated to a temperature of 60ºC by 60 minutes and to 63ºC by 60 minutes, finally the reaction was analyzed in the agarose gel 1.5% dyed with ethidium bromide. The result showed that the LAMP method detected a sample of Spinal Liquid as positive agreeing with the results obtained by means of cultivation. Conclusions, the development and application of the method of LAMP for the diagnosis of *P. aeruginosa* is a good advance in the medical area because it is known for being sensitive and specific, which allows to give a diagnosis and treatment in short time helping to prepare complications or deaths in infants.

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# Expression of the serine protease MarP from *Mycobacterium tuberculosis* in the periplasm of *Escherichia coli*

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Tuberculosis is among the most prevalent infectious diseases worldwide. This infection is caused mainly by *Mycobacterium tuberculosis*, a bacterium that has been difficult to eradicate because therapeutic treatments are long and current anti-tuberculosis drugs have led to the emergence of resistant strains. In addition, *M. tuberculosis* is capable of deploying a large number of defense mechanisms against the host immune system. The appearance of multidrug-resistant strains (e.g., resistant to two or more drugs) has generated the imperative need for new or improved therapeutic agents aimed at reducing the bacterial prevalence and thus the morbidity rate. To address this notion, it is necessary to identify essential proteins, including the factors involved in the virulence of the pathogen, which can be accounted as molecular targets for rational drug design. Among the latter is the serine protease MarP, encoded by the Rv3671 gene, which apparently plays an important role in the metabolism of *M. tuberculosis*. Furthermore, it has demonstrated that its expression is required for withstanding the harsh environmental conditions within the phagosome. Even more, gene suppression reduces the bacterial viability and affects the disease progression in the early and chronic phases. Interestingly, MarP has a disulfide bond that can be important for its native conformation; however, its dependence on a foldase, as the bacterial DsbA or DsbC, has not been tested. So, we have taken advantage of the availability of a bacterial model to examine the effect of the redox state on MarP. Here, we report the outcome of expressing MarP in the periplasm of three *E. coli* strains: one wild-type and two null-mutants (dsbA and dsbC). To express and target MarP to the periplasmic compartment, its nucleotide sequence was inserted in-frame with the pelB signal peptide and cloned under the control of the arabinose promoter. The efficient periplasmic expression was assessed by an immunoblot assay and its functionality is currently being evaluated by activity assays. We are engaged in demonstrating that the structure of MarP is essential for its physiological role, and its dependence on DsbA is crucial for *M. tuberculosis*.

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# THE IMPACT OF ADENOVIRAL PROTEIN E1B-55 kDa PHOSPHORYLATION ON THE DNA VIRAL REPLICATION

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During the replication cycle of human adenovirus type 5 (HAdV5) the reorganization of nuclei components is induced, this leads to the recruitment of cellular machinery that is responsible of viral gene expression to specific nuclear sites known as viral replication centers (RC), where viral DNA is located, and viral and celular proteins are recruited, these are responsable for replication, transcription and the start of pos-translational processing of viral genes. One of these proteins is the early region1B 55-kDa protein (E1B). E1B is a multifunctional phosphoprotein playing several critical roles during adenoviral productive infection, e.g., degradation of host cell proteins, viral late mRNA export, DNA viral replication and inhibition of p53-mediated transcription (Bridge E. et al 1990, Jimenez García , Ornelles S. 1991 and R. Gonzalez 2003 **.** Many of these functions are apparently regulated at least in part by the phosphorylation. The E1B protein is modified by phosphorylation on carboxyl terminal region S490, S491 or T495. Serines are phosphorylated by CK2 protein (W. Ching et. Al. 2012) and it has been suggested that threonine is phosphorylated by CK1.

Our results with mutant viruses in phosphorylation sites of the E1B shows that when it can not be phosphorylated in any of the three sites E1B is not localized RC associated, this also correlates with a defect in the formation of RC as well as the accumulation of DNA viral copies. On the other hand, when the phosphorylated state of the three sites is mimicked, RC are efficiently formed. The E1B association with these viral zones and DNA viral synthesis is efficient, and even before the process begins. Interestingly mutant viruses only affected on serines substituted by alanine, E1B remains associated to RC, viral DNA synthesis appears to have not a noticeable effect, but there is poor RC formation. Surprisingly in the mutant virus threonine 495/alanine, the phenotype is similar to the unphosphorylated E1B. In contrast, mutation of threonine 495 with aspartic acid results in the efficient association of E1B with RC, viral DNA synthesis and formation of RC are efficient compared with wt virus.

Something interesting is the fact that modifications by phosphorylation on E1B affects directly on the viral genome copies accumulation. This could be a direct effect on the mechanism of viral DNA synthesis or an indirect one.

These results show that the phosphorylation of E1B is necessary for its functions, and that the phosphorylation of threonine 495 is key to the functional state of E1B.

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# Study on the Antifungal Activity of Silver Nanoparticles

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Recently, silver nanoparticles (AgNps) have been shown efficient antimicrobial activity compare to other salts due to their extremely large surface area.[1] However, the antifungal effect of AgNps has received only scantly attention.[2] The AgNps were prepared using starch and cellulose as biopolymer matrices. This one-step method offers a simple and environmentally friendly approach to form stable colloids of nontoxic AgNPs. In this method a terminal aldehyde group of starch reduces the silver nitrate to silver metal and simultaneously stabilizes the nanoparticles in starch solution.[3] The AgNps were characterized by ultraviolet visible spectroscopy. In the current work the activity of the AgNps as antifungal was tested against different fungal species such as *Candida albicans, Candida krusei*, *Candida tropicalis* and *Colletotrichum sp.* Some characteristics known as virulence factors for Colletotrichum sp. genus, like: weight of the produced mycelium, sporulation, poligalacturonase activity and pH medium were evaluated during the growth of *Colletotrichum sp*. in three liquid medium commonly used for fungi culture. With the aim to use the AgNps as fungicide in the treatment of the anthracnose in *Annona muricata L*. giant type in the Tabasco state of Mexico.

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# Molecular characterization of *Gallibacterium anatis* -like strains harbored in hens

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*Gallibacterium anatis* is the etiological agent of gallibacteriosis an illness of egg producer hens. Vaccinated chickens and hens however could be infected with new strains, many of them non included in vaccines, suggesting a very narrow spectrum of immunity. To know about *G. anatis* strains harbored in discarded hens for egg production but commercially available for food consumption, we get four samples of five birds, these were dissected and inner organs were sampled for bacterial content inspection. Many colonies were recovered by culture on nutritive media. The main phenotype of pathogenic *G. anatis* strains is the hemolytic activity of gray small colonies on blood agar. Following the typical criteria forty field hemolytic strains were recovered and kinetics of hemolysin production was measured. Red cells from human and ovine were susceptible of hemolytic bacterial activity showing a higher production until five hours of incubation, and a reduction after that was observed. Fast characterization of twenty strains was made by DNA extraction and 16S rDNA amplification by PCR. Amplicons were sequenced by Sanger procedure and the nucleotide sequences were compared to GenBank references. One strain was 99% similar to *G. anatis* UMN179 and the other nineteen was related to uncultured bacteria, *E. coli* and *Shigella* sp. Surprisingly all new isolates have a reduced growth into nutritive media with rate similar to *G. anatis* and also have a kinetic hemolytic activity similar to pathogenic Gallibacteria. Genomic comparisons between these bacteria harbored in hens are in progress to found other similarities related to virulence and to distinguish the reasons for rare behavior of enteric-like bacteria.

Project of “RED de Modelos Microbianos de Interés Agrobiotecnológico”

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# Photobionts diversity is related with lichens ecological distribution in Guanajuato

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Lichens are symbiotic organisms formed by a fungus (mycobiont) and algae or cyanobacteria (photobiont), both partners contribute to lichen maintenance; the mycobiont provides protection while photobiont produces photosynthetic carbohydrates. The intimal dependence between these two members in some cases makes difficult they could live autonomously. However, some photobionts could be cultivated independently from its mycobiont partner. In order to establish the photobiont diversity from lichens from Guanajuato State. We isolated 8 different photobionts, and correlated its presence with the collected zone and lichen type. The methodological procedures involved serial growth in BG11 culture media and molecular characterization trough amplification and sequence analysis of *rbc*L gene. The genera found in our study included: *Myrmecia israelensis, Elliptochloris subsphaerica, Trebouxia sp.* and *Bracteacoccus minor*, all of them previously reported as lichen photobionts. The lichen diversity found in Guanajuato is correlated with their tolerance or sensitivity to atmospheric pollution, as nitrogen and sulfur dioxide, and this behavior is also allied with their photobiont member. From these isolated, we are be able to conduct symbiosis experiments using their mycobiont partner already isolated from our research group and start to study symbiotic regulation between both partners and the evolutionary state of the photobiont under fungus presence, and the regulation of secondary metabolites production in mycobionts when the photobiont is present.

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# Novel fusion proteins generated with fimbrial adhesins of uropathogenic *Escherichia coli*

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**Introduction.** Urinary tract infections (UTIs) are associated with high rates of morbidity and mortality worldwide. In Mexico, UTIs are a problem of public health, considered as the third cause of morbidity with approximately four million cases per year and uropathogenic *Escherichia coli* (UPEC) is the main etiology agent. Fimbriae assembled on the bacterial surface are essential for the adhesion in the urinary tract epithelium. In this study, FimH, CsgA, and PapG adhesins fused could be propose as a target for a vaccine designed to generate protection against UTIs.

**Methods.** The design of fusion proteins **was generated with bioinformatics tools and gene sequence was synthetized by GenScript. Fusion proteins expressions were induced in BL21 (DE3) *E. coli* strain using IPTG 1mM and His-tagged fusion proteins were purified by affinity chromatography using Ni-NTA resin under denaturing conditions (Guanidine 8M/Urea 8.5M). Fusion proteins were characterized into SDS-PAGE gels, Western blot, mass spectroscopy MALDI TOF/TOF, and dynamic light scattering (DLS). ELISA determined release of IL-6 and IL8 cytokines, and antibodies in the sera and urine.**

**Results. A template gene with the following order** *fimH-csgA-papG-fimH-csgA* (*fcpfc*) linked with the nucleotide sequence that coded for the $[EAAAK]_5$ peptide was synthetized. **Monomeric (*fimH*, *csgA*, and *papG*), dimeric (*fc*), and trimeric (*fcp*) genes cloned in the pLATE31 expression vector showed products of** 1040, 539, 1139, 1442, and 2444 pb**, respectively.** *SDS*-PAGE *gels staining with Coomassie* Blue **and Western blot analysis of monomeric, dimeric, and trimeric proteins displayed bands of 26.5, 11.9, 33.9, 44.9, and 82.1 kDa that correspond to** FimH, CsgA, PapG, FC, and FCP, respectively. **The** protein concentrations for FimH, CsgA, PapG, FC and FCP proteins were of 2.7, 0.697, 2.63, 1.03, 0.998 mg/mL, respectively. **Mass spectrometry analysis by MALDI-TOF/TOF revealed the presence of specific peptides that confirmed to the fusion proteins.** A polydisperse state of fusion proteins by DLS analysis was determined. FimH, CsgA, and PapG proteins stimulated the IL-6 releases from 372 to 398 pg/mL; interestingly, FC and FCP proteins stimulated the IL-6 release of 464.79 pg/mL ($P \leq 0.018$) to 521.24 pg/mL ($p \leq 0.002$), respectively. In addition, FC and FCP proteins stimulated the IL-8 release of 398.52 pg/mL ($p \leq 0.001$) and 450.40 pg/mL ($P \leq 0.002$), respectively. ELISA using the fusion protein showed high levels of IgA and IgG antibodies in the human sera; however; under same conditions low levels of IgA and IgG antibodies in human urine were detected.

**Conclusion**. FC and FCP proteins were high stable, with antigenic properties and with ability to induced releases of **IL-6 and IL8 cytokines.**

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# Functional analysis of *RSP_1318* a component of the Fla2 flagella of *Rhodobacter sphaeroides**

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Bacteria possess different mechanisms that respond to the conditions of the environment. One of them is the ability to move toward favorable environments. Chemotaxis is one type of taxis that modulates the direction of rotation of the flagellum that is used for the displacement in liquid and solid surfaces. The bacterial flagellum is a supramolecular complex composed of at least 30 different proteins and the structure consists of three parts the filament, which is an helicoidal structure that can reach 15 µM of length and serves as helical propeller; the hook, which connects the basal body to the filament and allows torque transfer to the filament; and the basal body, this structure is composed of various ring structures (P, L, MS and C), the rod, the export apparatus and the stator complex.

In *Rhodobacter sphaeroides* two sets of flagellar genes coexist, *fla1* and *fla2*. *fla2* correspond to a set of flagellar genes of the bacterium and is expressed when the flagellar transcriptional regulator *fleQ* of *fla1* is interrupted. The function of many genes of the *fla2* set are still unknown therefore the objective of this work was to study the role of Open Reading Frame (ORF) annotated in the genome as *RSP_1318*. This ORF is part of an operon composed by four more genes that include *fliL2*, *motA2* and two genes of unknown function. The mutation of this ORF produces a *mot* ¯ phenotype given that mutant bacteria show intact flagella. Moreover, given that this protein is located in the periplasm, it could be interacting with stator complex. These results suggest that this protein could be part of the stator, which is responsible of coupling the proton motive force with flagellar rotation.

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# Study of essential genes in *Escherichia coli*.

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The increased availability of genome sequences has provided the basis for comprehensive understanding of organisms at the molecular level. *Escherichia coli* K-12 has been one of the best-characterized organisms in molecular biology and whole-genome sequences are now available for many strains of *E. coli.* In order to understand phylogenetically the genomic information has been classified in three major classes. The core genome, that is formed by all the DNA sequences that are conserved among all individuals of a species, and is proposed to include the genes responsible for the basic aspects of the biology of a species and its major phenotypic traits. By contrast, dispensable genes or accessory genome, is defined by genes that can be present in a part of the individuals of a species and is thought to 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 “species”: core genome + dispensable genome. Even though it has been defined that the essential genes are in the core genome, and that microorganisms inherit a genome from their parent cells that reflects their phylogeny, it was recently demonstrated in *Azotobacter vinelandii* that genes, which define its biology, were acquired by HGT*.

The *E. coli* Keio mutant collection lacks mutants in 303 genes, that are candidates for essential genes, in order to find genes with essential functions, that are not part of the core genome, we analyzed the presence of the 303 “essential genes” in 63 complete genomes of *E. coli* with amino acids sequences and we selected some absent genes. We found 67 genes with anomalies; some were absent in only one strain, and appear to be involved in fundamental cellular processes. We selected the genes *bcsB* (bacterial cellulose biosynthesis) and *dxr* (1-Deoxy-D-xylulose 5-phosphate reductoisomerase) from *E. coli* commensal HS and uropathogenic CFT073 strains, respectively, to evaluate whether these gene were really essential. We will present evidence of the essentiality of the gene *bcsB,* according to its biological function and discuss the value of the available genome sequences as a resource for the research of bacterial evolution.

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# Involvement of cyclodipeptides in the bacterial population control of the Churince system of Cuatro Ciénegas basin

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The Cuatro Cienegas Basin (CCB) is an aquatic ecosystem extremely oligotrophic with low nutrient content, but dominated by diverse microbialites. In this site, some ponds are abundant or dominated by the *Pseudomonas* linage, such as the Churince system. The *Pseudomonas* genus has been described to possess the capability to produce a wide variety of virulence factors and utilizes uncommon adaptative metabolic pathways to compete in its ecological niches. Cyclodipeptides (CDPs) are non-ribosomal cyclic peptides which have been proposed as signal molecules utilized in the bacterial population control, these are commonly produced by diverse microorganism and possess antimicrobial, antifungal and antiviral properties. In this work, we determined the CDPs production in a bacterial population isolated from the aquatic system Churince in CCB and studied the effect of these compounds over the population control. Results indicated that the CDPs were produced for the major of bacterial isolates and were associated with their antagonistic property, suggesting that these compounds are excreted to eliminate competition in this ponds with scarce nutrients, prevailing the *Pseudomonas* communities with ability to control the growth of other bacterial populations. Further, the antibiotic DAPG was produced in several isolates suggesting that also is involved in bacterial population control. These results show that the CDPs and DAPG produced by the *Pseudomonas* communities are involved in bacterial population control and dominance by an antibacterial mechanism independent of its quorum sensing signaling.

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# Role of the nucleoid-associated proteins H-NS and StpA in the regulation of *Citrobacter rodentium* virulence.

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*Citrobacter rodentium* is a mouse bacterial pathogen used as a surrogate model to study the pathogenesis of attaching and effacing (A/E) pathogens, which include the human pathogens enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC). The ability to cause A/E lesions in the intestinal epithelium of their respective hosts is conferred by the locus of enterocyte effacement (LEE), a pathogenicity island encoding a type III secretion system (T3SS) and several effector proteins. The nucleoid associated protein H-NS acts as a global regulator repressing the expression of many genes, particularly those involved in bacterial virulence that have been acquired by horizontal transfer events; whereas StpA is an H-NS-like protein with RNA chaperone activity that may act as a molecular backup for H-NS and form heterodimers with it. In EPEC, H-NS strongly silences LEE gene expression, which is in turn positively regulated by the H-NS antagonist Ler, an H-NS paralog encoded within the LEE. In addition to H-NS, StpA and Ler, *C. rodentium* expresses two other H-NS homologs named H-NS2 and H-NS3. Ler is essential for LEE gene derepression as in EPEC, but the interplay of the other H-NS-like proteins in LEE regulation has not been characterized yet.
In this work, we show that type III secretion (T3S) is enhanced in both the *hns* and *stpA C. rodentium* mutants under repressing growth conditions, but is unaffected in the absence of H-NS2 or H-NS3. As expected no secretion was detected for the *ler* mutant. This observation was confirmed by western blot using antibodies against EspB, a translocator protein encoded in the *LEE4* operon, and EscJ, a T3SS structural protein encoded in the *LEE2* operon. In agreement with the secretion profiles, the expression of both EscJ and EspB was stimulated in the absence of StpA and H-NS, thus confirming that both proteins negatively regulate LEE expression. Deletion of the *ler* gene in the Δ*stpA* and Δ*hns* backgrounds abolished T3S and EspB and EscJ expression even under inducing growth conditions, indicating that Ler is required to overcome the repression exerted by both repressors. Moreover, overexpression of an H-NS dominant negative mutant in the Δ*stpA*Δ*ler* restored T3S and LEE protein expression under repressing growth conditions. Despite having a reciprocal function in the regulation of the LEE, the *hns* mutant showed growth defects and was unable to colonize mice, while the *stpA* mutant resembled the wt strain in both assays, indicating that H-NS plays a major pleiotropic role in *C. rodentium* in contrast to StpA. In summary, these results revealed that in *C. rodentium* StpA and H-NS play an additive role in LEE negative regulation; however, in the absence of Ler both repressors can functionally replace each other to fully repress LEE gene expression. It remains to be determined whether StpA and H-NS independently bind to overlapping binding sites on LEE promoter regions or form heterodimers to efficiently control LEE gene expression when both proteins are present. *This work was supported by DGAPA IN209713 and CONACyT 154287, 239659*

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# *Expression of the sRNA´s CrcZ and CrcY in Different Carbon Catabolic Repression Conditions in Azotobacter vinelandii*

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**Introduction.** Carbon Catabolite Repression (CCR) allows the bacteria to selectively assimilate one compound among a mixture of potential carbon sources, which facilitates growth and survival in different habitats as well as the adaptation to changing environmental conditions. This is achieved by regulating the expression of genes involved in the uptake and assimilation of non-preferred compounds (Rojo, 2010). In *Pseudomonas spp.* the post-transcriptional regulatory system CbrA/CbrB system control the expression of the s*mall non-coding RNAs (sRNA´s),* which modulate the activity of the catabolite repression control protein Crc, enabling the utilization of the less prefer carbon source (Sonnleitner *et al*., 2009). *Azotobacter vinelandii* is an organotrophic, nitrogen-fixing gamma proteobacteria phylogenetically closed to *Pseudomonas* that prefers the use of organic acids to carbohydrates such as glucose as carbon source (Segura *et al*., 2014).

**Background.** With the aim of investigating the molecular mechanisms underlying carbon catabolite repression by the CbrA/CbrB system in *A. vinelandii,* transcriptional fusions of the *crcZ* and *crcY* regulatory region with the *gusA* reporter gene were constructed. In this study we are investigating the dynamic in the regulation of CrcZ and CrcY expression under different CCR conditions; for this, we evaluated the sRNA´s expression in acetate (strong CCR), glucose (medium CCR) and in a mixture of these carbon compounds.

**Results.** In *A. vinelandii* we found that the expression of CrcZ and CrcY showed a similar pattern between them under different CCR conditions. However, their expression markedly differed in cells grown in acetate in relation to glucose grown cells. In a good carbon source like acetate, the promoter activity was not detected in exponential phase. The opposite case was detected in the less prefer carbon source glucose, where the promoter activity was detected while carbon source was being consumed, after that the promoter activity presented a sustained increase until stationary end phase. In acetate-glucose cultures the dynamic in the promoter activity was similar than in sole carbon source cultures; low activity was detected when acetate is being consumed and it increased when glucose is being consumed. These results confirm that the expression the sRNA´s CrcZ and CrcY is related to the carbon and energy source preference in *A. vinelandii*, and as in other *Pseudomonas* species their levels determine the strength of CCR. We are currently investigating the effect of fixed Nitrogen, a condition known to relieve CCR, on the expression of CrcZ and CrcY.

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# Study and identification of cold adaptation proteins in the Antarctic yeast *Rhodotorula mucilaginosa*

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Above 80% of the Earth's environments has temperatures below 5°C, under these extreme conditions exist a great biodiversity, which includes some yeasts species.

Organisms that develop at low temperatures have a set of adaptive mechanisms that enable them to cope with such conditions. In yeast, responses against cold shock have been extensively studied in *Saccharomyces cerevisiae* where it was observed that there are three stages of response to moderate cold shock (10 - 18 ° C). In the early stage, genes related with transcriptional machinery and ribosome biogenesis are induced. In the middle stage, genes involved in maintaining cell wall and with translational machinery are overexpressed. In the late stage, genes of step 1 and 2 are inhibited, while those related with cellular metabolism and signal transduction are induced. Furthermore, a transcriptional activation of typical stress markers is induced. Cryoprotectants synthesis, such as trehalose and glycerol, are induced against cold shock below 10°C, at near freezing temperatures.

Other important proteins in adaptive response to cold-stress induced are antifreeze proteins (AFPs) which inhibit ice recrystallization. These have been reported only in three basidiomicetous yeast species.

In our group, we are interested on understanding the cold adaptation mechanisms in the Antarctic yeast *Rhodotorula mucilaginosa*. Whit this objective, we try to identify genes involved in this process. It is planned to clone some of them in *S. cerevisiae*, and evaluate changes on it´s cold adaptation. Proteins selection will be performed by BLAST against reported proteins involved in cold adaptation and cold shock induced in other yeasts.

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# Clonning and expression of Dengue virus protein NS4A

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Introducction: Dengue virus is the most important arboviruses with a health public impact worldwide. The genome of this virus encodes structurals and Non-structurals proteins, this proteins are involved in architecture of virus and replication respectively. Several studies has identified that NS4A protein of Dengue virus has a function important in the production of viral replication complex, has been shown that NS4A protein induced structural changes in the membranes of endoplasmic reticulum, this new structures are relevant to generate a microenviroment necessary for ensambly and viral replication. The recombinant protein producction is a strategy for studies of interaction with cellular factors, functional biochemestry and antiviral desings, for this reasons in the present work we clone and expresed N4A of Dengue virus.

Material and Methods: The first step was amplified the sequences of NS4A, this sequences was cloned in a bacteria expression sytem. Later an induction kinetic with IPTG was perfomed, and them the expression of NS4A protein was evaluated by western blot using an antibody against histidine tag. Finally the recombinant protein NS4A, was purified from bacterial inclusion bodies by preparative gels and its purity was determined by staining blue comasie, and western blot.

Results: The sequeces NS4A of DENV was cloned in prokariotyc vector. The analysis of the kinetics induction showed that NS4A had a better expression at 12 hours post-induction, to identify at this time a band of 17 kDa which is the predicted weight of NS4A in the western blot conducted. Finally a 17kDa protein was detected by western blot assays and coomassie blue staining of the inclusion bodies purified by preparative gels.

Conclusion:, The data obteined in this work, Showed that bacteria system is a good tool for expression and production of NS4A protein. This proteín was expressed in bacterial inclusion body, and a first strategy of purification the NS4A protein was isolation this structures and purified NS4A used preparative gels. In the future this protein will use for inmunogen and produce a polyclona antibody. In addittion new strategys of purification will employed for biochemical assays.

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# Characterization of the quorum-sensing system RhlR/RhlI of a *Pseudomonas aeruginosa* dolphin isolate.

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*P. aeruginosa* is a ubiquitous and versatile bacterium capable of producing acute and chronic infections in humans due to the production of several extracellular virulence factors. The expression of those virulence determinants is regulated by the so called mechanism *quorum sensing response* (QSR).

The *P. aeruginosa* QSR is comprised by the LasR-LasI and RhlR-RhlI systems. LasI and RhlI are acyl-homoserine lactone synthases producing 3-oxo-dodecanoyl homoserine lactone (3OC12) and butanoyl homoserine lactone (C4) respectively; while LasR and RhlR are transcriptional regulators that after binding to their cognate signals activate target gene expression. Since LasR controls the expression of both *rhlR* and *rhlI*, the Las system is considered to be at the top of the QSR hierarchy.

Strain 148 of *P. aeruginosa,* isolated from a dolphin, lacks the Las system but is still virulent in a mouse-based model and does produce RhlR-dependent virulence factors such as pyocyanin and rhamnolipids.

The aim of the present work is to determine how the expression of the Rhl system in this strain is activated independently of LasR and how the production of the RhlR-dependent virulence factors is regulated.

# Fungal endophytes diversity associated to *Pytogramma sp.,* thermotolerant fern from "Los Azufres", Michoacán.

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Plants respond to abiotic stress (high temperature, salinity, desiccation) through a complex signaling system involving the perception of signal transduction by means of signaling pathways, followed by genetic and physiological responses, either through the production of osmolytes, altering water transport and by sequestering reactive oxygen species. However, 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 particular type of plant is influenced by various factors such as geographic location, weather patterns, plant physiology and tissue specificity. This work aims to isolate and characterize endophytic fungal a fern (*Pityrogramma* sp.) able to develop in the geothermal area of "Los Azufres" in Michoacan, México. Plant develops near geothermal fumes and is able to withstand temperatures up to 50 °C in the root zone. In order to obtain endophytic axenic cultures, standard microbiological methods were used, the identification of the isolates was performed by their macroscopic and microscopic characteristics. 79 endophytic fungi were isolated from root and leaf of *Pityrogramma* sp., these come from three independent sampling places in which were obtained 27, 25 and 27 isolates respectively. Genera as *Penicillium* sp., *Aspergillus* sp., *Trichoderma* sp., *Cladosporium* sp., *Cladophialophora* sp. and *Bipolaris* sp, were found as the principal fungal endophytes associated to this plant. Molecular identification of fungal endophytes was based on sequence analysis of ITS1 genomic region. The results showed that *Pityrogramma* sp. maintains a symbiotic association with these fungal endophytes. It remains an open question about this fungal endophytic community are related with the thermotolerance found in its host. Nevertheless, we conclude that these fungal endophytes should be considered with a promising potential to be probed in other plants of economic importance to improve tolerance to abiotic stress.

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# Association between *Helicobacter pylori* infection and periodontitis.

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*Helicobacter pylori* is a bacterium known to be the cause of diseases like chronic gastritis, peptic ulcer and more important, gastric cancer, this infection has a higher prevalence in developing countries such as ours, the transmission mechanism of *H. pylori* is not yet completely understood. It has been reported that this bacterium can be found in saliva, dental plaque, gingivocrevicular fluid and other sites of the oral cavity. Interactions with other bacteria may help *H. pylori* to survive in this environment, for example it has selective unions with dental plaque bacteria like *Fusobacterium nucleatum* and *Porphyromonas gingivalis*, also dental plaque provides bacteria with an increased defense against antibiotics and the immune system. Periodontitis is an inflammatory disease originated from pathogenic bacteria in dental plaque and a sustained immune response from the host, as a result of this disease, periodontal tissue is destroyed creating periodontal pockets and if the disease goes untreated teeth loss may occur. International articles have reported that *H. pylori* is present in dental plaque of patients with periodontitis in this study we evaluated the association between *H. pylori* and periodontitis in a mexican population. We evaluated the presence of *H. pylori* in dental plaque samples from 47 patients with periodontitis and 53 samples from a periodontally healthy group; we used PCR as the detection method with primers for the *glmM* and *16SrRNA genes*. Our results showed a total prevalence of 11% (n=11) and absence of *H. pylori* in the periodontitis group OR 0.3890 IC 95% 0.002224 to 0.6803 therefore we concluded that *H. pylori* can be found in the oral cavity but has no association with periodontits.

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# Endophytic fungus with antibiotic activity against fungal plant-pathogens

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Filamentous fungi are well known for producing substances with antimicrobial activities, several of which may have potential agricultural, environmental, and pharmaceutical use. Novel natural products with antimicrobial activities have been isolated from endophytes; some of these compounds are antibiotics that have antifungal, antibacterial or insecticidal properties. The aim of the present study was to isolate endophytic fungi with antimicrobial activity against fungal plant-pathogens. Samples were obtained from roots of native plants growing at San Quintin Valley (Ensenada, Mexico). From 30 obtained fungi, an isolate named BCRSQ6-1 showed *in vitro* antagonistic activity against *Macrophomina phaseolina* and *Botrytis cinerea,* with inhibition growth percentages of 56% and 84% respectively. BCRSQ6-1 releases non-volatile compounds that fully inhibited the growth of *Botrytis*, while in *Macrophomina* the inhibition is slight, but the formation of microsclerotia was diminished. Regarding the morphology, colony on PDA is velvety, white, green olive at center of the reverse of the plate. Colony on MEA is velvety, white, without change over age; mycelium immersed, without visible pigments. Hyphae are hyaline. After twenty days, small, white and spherical clumps appear on the surface of colonies. Later they become black dots that under the light microscopic consist of aggregates of chlamydospore-like cells, of around 4 µm of diameter. These characteristics indicate that the fungus belongs to the Chaetomiaceae family. Analysis of the PCR-amplified sequences: ß-tubulin, EF-1α, and Actin, rendered hits with low percent of similarity, being the highest with ß-tubulin (85%) for *Chaetomium cuniculorum*. Since members of *Chaetomium* are strongly cellulolytic, and in BCRSQ6-1 this activity was not detected, the isolated obtained here seems to be a novel fungi with antibiotic activity.

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# Analysis of the effect of nucleolin and CTCF in adenovirus 5 replication

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Adenoviruses are non enveloped, double stranded DNA viruses that cause 5 to 10% of the acute respiratory infections in infants worldwide. During the viral replication cycle cellular components of the nucleus that are responsible for transcription regulation, cell cycle control, DNA damage and antiviral response are relocalized to specific sites that form ring-like structures called Replication Compartments (RC) where the viral genome is expressed and replicated.

We have recently found that nucleolin, a major component of the nucleolus that participates in transcription and processing of rRNA and may have a role as a scaffold of this cellular domain is relocalized to the periphery of adenovirus RC. In addition, a highly conserved regulator of transcription and chromatin structure, CCCTC binding factor (CTCF) is known to associate with RC and impact adenovirus replication. The role of these essential proteins during the replication of the virus is not known. In this work, we have used the CRISPR-Cas9 system to knockout *ncl* and *CTCF* gene expression in primary human cells to analyze the effect that these proteins may have on the replication cycle of adenovirus in primary human cells.

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# Isolation and characterization of microorganism with agronomic importance from the rhizosphere of "maguey pulquero" (*Agave salmiana*)

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Abstract

Rhizosphere, the interface between the plant roots and soil microorganisms, is a biochemically complex environment which support the development of diverse microbial communities. Recent advances in microbiological and molecular techniques, has unravelled the importance of rhizosphere resident for soil fertility, plant growth promotion, supression of phytopathogens, and improve agronomic yield of crops.

In order to research the rhizosphere microbiome identity and its role on maguey pulquero (*Agave salmiana*) in the tolerance to extremes conditions, we sampled Agave´s roots of three regions from Hidalgo. The bacterial population was identified by total DNA extraction and subsequent 16S rRNA sequencing. The microbiological analysis on selective culture media, showed strains from the genera: *Pseudomonas*, *Bacillus*, *Azospirillum* and *Rhizobium*, all of them valued for their activities in the nutrition and health of plants.

The native strains will be apply to sterile soils, for search its potential to contribute in the development of sustainable agricultural systems. We consider very important that the use of plant growth promoting microorganisms for reduce chemical fertilizer and pesticides, come from the same region where will be used, this for taking advantage of its biotechnological applications in the development of ecofriendly sustainable agriculture.

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# Novel methodology to fractionate cell proteins for two-dimensional gel electrophoresis

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Introduction: The proteome analysis (proteomics) is the complete study of the proteins present in a sample. The purpose of proteomics is to determine the presence, relative abundance, and the status of posttranslational modification of proteins, thus generating a very informative image of the state of a cell. One of the biggest challenges of the proteomic analysis is the reproducible separation of complex mixtures of proteins, and the two dimensional electrophoresis (2DE) is the only wide scope tool based in its quality to distinguish among hundreds or thousands of proteins, because it separates proteins based simultaneously on the isoelectric point and the molecular weight. However the lower the mixture complexity the higher the 2DE resolution, for example the identification of a single protein using mitochondrial extracts is easier than using total extracts. In the case of membrane proteins, they should be solubilized in low ionic strength and without compounds that change the net charge or the size. Many methods of separation of membrane proteins are based on the extraction with ionic detergents thus being incompatible with 2DE, furthermore their extraction principle depends on the hydrophobicity and not on the actual location of proteins. The goal of this work is to generate a strategy to fractionate the membrane, cytoplasmic and nuclear proteins based in their localization and maintaining its compatibility with 2DE.

Strategy: Subconfluent cultures (90%) of MDCK and HMEC-1 cells were used in 25$cm^2$ culture plates. Cells were detached at 37°C without mechanical stress and without trypsin, in order to preserve cell integrity, and were harvested by mild centrifugation. They were washed with isotonic solution. Then they were fast frozen in liquid nitrogen to modify plasma membrane permeability. Frozen cells were lysed with hypotonic solution without detergents. Afterwards they were harvested again to recover the cytoplasmic fraction (supernatant). Nuclei and remaining membranes (pellet) were lysed with hypertonic solution without detergents and were centrifuged at high speed to recover nuclear proteins. The membranes (pellet) were washed again with hypotonic solution an centrifuged again at high speed. The so cleaned membranes were dissolved in a concentrated sucrose solution and summited to ultracentrifugation. The soluble fraction was discarded and the insoluble fraction was dissolved with non-ionic detergents and chaotropic agents. Protein concentration was determined with Bradford reagent. The protein content analysis was made by conventional electrophoresis and 2DE in the presence of reductive agents and ampholytes and was revealed with Coomasie Blue stain or Silver stain. The presence of proteins belonging to each fraction was evaluated by Westernblot.

Results: An enrichment of proteins of each fraction and an impoverishment effect of associated contaminants (proteins not belonging to that fraction) compared to a separation based on hydrophobicity method was observed.

Conclusions: This work presents a simple methodology of membrane, cytoplasmic and nuclear protein extraction from the same sample and compatible with 2DE

# Neutralizing activity of anti-M1 antibodies against equine influenza virus

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**Introduction:** *Influenza A virus* is a significant and persistent problem of human and animal health worldwide. It has the potential to generate antigenic variants that could escape to the protective immunity conferred by vaccination or prior infection. A strategy for expanding control over the virus is to develop heterosubtypical immunity through the most stable proteins of the virus. In this study the neutralizing capacity of anti-M1 antibodies was determined. **Methodology:** Samples were obtained during January to April 2015. A total of eight samples of nasal fluid and 123 samples of serum were obtained from horse-drawn trash service in metropolitan area of Monterrey, Nuevo Leon, Mexico. All nasal fluids were tested by RT-PR using primers recommended by WHO for typing M gene of influenza virus A and serum samples were tested by ELISA. In addition, the matrix gene from *Influenza A virus* (H1N1/09) was cloned and expressed and polyclonal antibodies anti-M1 in rabbit were produced to determine neutralizing activity of both anti-M1 antibodies and sera collected from horses**. Results:** A fragment of 244 bp of M1 gene was amplified in 6 (75%) nasal fluids, while 114 (92.68%) of the sera tested showed reactivity against M1 protein (H1N1/09). In addition, polyclonal antibodies anti-recombinant M1 and a horse serum showed neutralizing capacity in both vaccine strain and *Influenza A virus* obtained from nasal fluids. **Conclusions:** These results suggest that M1 protein could generate antibodies that confer protective activity against Influenza viruses circulating in this animal population.

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# Searching for the function of an early protein from bacteriophage PaMX41 infecting *Pseudomonas aeruginosa*.

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An important challenge in the post-genomic era in bacteriophages research is the functional study of the increasing amounts of hypothetical genes that are abundant in newly sequenced genomes of phages. Genes expressed at early times of phage infection are associated with hijacking the host molecular machinery to create the right environment for the phage propagation. These genes are among the less conserved in phages as they usually have no match in data bases. The podophage PaMx41 has been studied in our group at the genomic and transcriptional level. The early ORFs lack homology, at the nucleotide, amino acid and structural levels, to any other characterised protein. ORF2 is one of the early ORFs whose mRNA was observed transiently during the first 15 minutes of infection, unlike most of the phage mRNAs present from 15 minutes-on that remained until the end of infection.

In order to investigate the function of ORF2, we cloned it in the expression vector pPROEX-1, which adds a 6xHis-tag to the expected protein. Overexpression of ORF2 had no effect on the growth of *E. coli*, the heterologous expression of the recombinant protein was observed by SDS-PAGE and Western blot and was purified on Ni-Agarose columns. Using the purified protein, pull-down assays will be carried out to identify the interactions with proteins of the *P. aeruginosa* host and those expressed by the phage during lytic cycle. Finally, we will clone ORF2 in the shuttle vector pHERD30T to analyse the physiological effect of the phage protein in the host strain. This results will help to elucidate the function of the ORF2 during early stage PaMx41 infection.

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# The use *Killer's* toxin from yeast *S. cerevisiae* as an inductor of cellular death in microorganisms with biomedical importance

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Some strains of *Saccharomyces cerevisiae* secrete toxins named *killer* (*K1*, *K2* and *K28*), for their lethal effect in sensitive strain. The producers are denominated $K^{ill+}$ and, strains unproducers $K^{ill-}$ (sensitive to *K1*). Subsequent to *S. cerevisiae* genome sequencing, the channel TOK1 was recognized as *K1* receptor. In yeast $K^{ill-}$ the *K1* induce channel activation, increase the open probability, and then cells are depleted of potassium ions, this promotes a membrane depolarization and cell death. Interestingly, $K^{ill+}$ strains are protected of this effect by the action of an immature toxin (*pptoxK1*), localized in intracellular TOK1 structure. In this way, *S. cerevisiae* present a dual toxicity-resistance system, to study mechanisms of interaction at molecular level.

Recent evidence indicates that other organisms as *Candida*, *Aspergillus* and *Neurospora*, expressed homologous protein like TOK1, suggesting that dual toxicity-resistance system are conserved in the evolution.

The aim of this study was determine the toxin *killer* effect in microorganisms of biomedical importance.

Results obtained from *in sillico* analyses shown that homologous genes to TOK1 are present in *Klebsiella pneumoide*, *Staphylococcus aureus* and *Listeria monocytogenes*. Microbiological studies indicates that *K. pneumoide*, *S. aureus* and *L. monocytogenes* recognized *K1* toxin from *Saccharomyces cerevisiae*, this data suggest that a possible interaction of the *K1* toxin in these prokaryotes.

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# Analysis of the disulfide oxidoreductase activities of a DsbA-like protein from *Mycobacterium tuberculosis*

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Protein function depends mainly on its native structure, which is achieved through a cellular process named protein folding. During folding of secreted proteins, correct disulfide bond formation is an important post-translational modification (PTM) assisted by oxidoreductases. In bacteria, this PTM is catalyzed by Dsb family members, being DsbA the major oxidase and DsbC the main reductase/isomerase. Recently, a DsbA-like protein has been identified in *M. tuberculosis*, named Mt-DsbA, which is essential for optimal growth of this pathogen. Furthermore, since Mt-DsbA is anchored to the cytoplasmic membrane, it has been suggested that might be involved in oxidative folding of secreted proteins, as nearly 60% of them require proper disulfide bond formation. Therefore, for considering Mt-DsbA as a molecular target for anti-tuberculosis drugs, the characterization of the enzymatic activities is required to establish its functional role in the physiology of *M. tuberculosis*. Here, we report the molecular cloning of the fragment encoding the soluble region of Mt-DsbA, the heterologous expression in *E. coli*, the purification of the recombinant protein under native conditions, and the characterization of its oxidoreductase activities. By standard enzymatic activity assays using non-physiological polypeptides as substrates, we found that Mt-DsbA exhibits a limited oxidase activity and lacks reductase activity, suggesting that its oxidoreductase activities might be limited to specific physiological substrates with a reduced number of disulfide bonds. Therefore, to gain further insights into its functional role is imperative to perform enzymatic assays using physiological substrates, as the serine protease Mt-MarP, to establish the potential of Mt-DsbA as a target for search and design of novel drugs with therapeutic activity against *M. tuberculosis*. Studies on the oxidative folding of Mt-MarP and its dependence on Mt-DsbA are in the process of being addressed.

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# Types of melanin produced in the grapevine phytopathogenic fungus *Lasiodiplodia theobromae*

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In some phytopathogenic fungi, melanin is an essential compound for the development of pathogenicity. Melanin is a pigment produced by the oxidation of phenolic and indole compounds. There are different types of melanin depending of their precursors. *Lasiodiplodia theobromae* is a fungus belonging to the Botryosphaeriaceae family and one of the most aggressive pathogens in grapevine which cause degenerative diseases, dieback and plant death. In this fungus, melanin is deposited in the intern surface of cell wall, but its participation in the process of infection of vid is unknown. The search of factors involved in the pathogenicity of vascular pathogens is important to understand their interaction with the plants they infect. The aim of this work was to study the behavior *in vitro* of *L. theobromae* str. UCD256Ma in the presence of melanin inhibitors and under different stress conditions. First, fungus was grown in the presence of inhibitors to DHN-melanin (tricyclazole and phthalide), DOPA-melanin (tropolone and kojic acid) and pyomelanin (nitisinone); next, the effect of the combination of these inhibitors with different types of stress (enzymatic lysis, $H_2O_2$, and UV radiation) was done; and last, the ability of the fungus to use tyrosine as carbon and nitrogen was evaluated. The fungus has the ability to grow using tyrosine as carbon or nitrogen source. In the presence of tropolone at 15 $\mu g \cdot mL^{-1}$ the growth of *L. theobromae* was inhibited and the fungus lost the ability to degrade tyrosine. The exposition to $H_2O_2$ only affected the development of *L. theobromae* in the presence of 15 $\mu g \cdot mL^{-1}$ of tropolone. A total growth inhibition was observed with the combination of an enzymatic extract of *Trichoderma asperellum* and 15 $\mu g \cdot mL^{-1}$ of tropolone. Finally, viability was affected only in non-melanized spores when they were exposed to UV radiation. According to this results, *L. theobromae* str. UCD256Ma can use the tyrosine as carbon and nitrogen source for its development and probably as a precursor of melanin; the melanin protects the fungus against abiotic stress conditions and although the fungus produced three types of melanin, its principal is DOPA-melanin, which is involved in the production of aerial mycelia.

# Genetic and Antigenic relation of VP8* and VP5* subunits of VP4 protein of Rotavirus on Northern Mexico

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**Introduction.** Rotavirus is the main cause of viral gastroenteritis around the world.
Globally, P[8] genotype  Globally, the genotype P8 is the most incidence in combination with other G genotypes. Nine antigenic regions have been established on VP4 protein that are responsible to elicit neutralizing antibodies. Four of these regions have been mapped on VP8* subunit (8-1 to 8-4) and five on VP5* subunit (5-1 to 5-5). Therefore polyclonal antibodies elicited against VP8* subunit of P[8] genotype were evaluated and analyzed in field rotavirus. **Methodology.** In this study 76 positive samples of rotavirus P[8] genotype were selected.  The antibody reactivity was evaluated by ELISA. The VP8* and VP5* regions of VP4 gene of three positive and one negative samples by ELISA were cloned and sequenced. **Results.** In this study, 55 (72.3 %) samples were recognized by antibodies anti- VP8* subunit of P[8] genotype. Sequences analysis of three of these positive samples and one negative sample revealed 10 variations in the VP4 protein, 3 in VP8* and 7 in VP5*.
On the other hand, 2 of 3 positive samples showed 100% identity in the region of VP8* when were compared with the negative sample. However, two amino acid changes (N738T, N766I) with possible structural implication were detected in VP5 subunit. **Conclusions**. This results suggest that amino acid substitutions in VP5* subunit could be implicated in the lack of antibody recognition of VP8 subunit.

# Expression of HBD-2 in patients with viral infections and asthmatic crisis

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**Background:** Asthma is a chronic obstructive disease, characterized by obstruction, hyper-responsiveness and inflammation that affect people worldwide. Viral respiratory infections are the major cause of asthma exacerbations. One of the mechanisms implicated in this association are the β-defensins. They are cationic peptides with antiviral activity which recently have a demonstrated capacity to modulate innate and adaptive immunity. **Objectives:** To determine the expression of the HBD-2 in epithelial cells and serum of patients with viral infections, with asthmatic crisis and without asthmatic crisis. **Material and Methods:** Two groups of patients were studied: patients with diagnosis of asthma crisis, and patients without asthma crisis that were attended in INER (2013-2014). In nasopharyngeal epithelial cells is detected the respiratory viruses and HBD2 expression was detected for RT-PCR. A Spirometry was performed and the concentration of HBD2 in blood was determined by ELISA, as well as the immunoglobulin IgE and inflammatory cells counts. **Results:** 25 patients with asthmatic crisis were studied from 18 to 61 age years. Patients without asthmatic crisis were 36 from 22 to 80 age years. The lung function was measured (FEV1%) and the patients were classified by asthma severity. The concentration of immunoglobulin IgE in the patients with asthmatic crisis was from 20.8 to 3100 U/ml and patients without asthmatic crisis were from 5 to 9,430 U/ml. The neutrophil was the cell more abundant. The patients with asthmatic crisis solely presented viral infections (92%) and the viruses more frequent were IA (88%) and RV (52%). The expression of hBD2 in epithelial cell in patients with asthmatic crisis was 56% y without crisis was 67%. The concentration in serum of HBD2 in patients with asthmatic crisis was from 842 to 58,026.5 pg/ml (20%) and in patients without asthmatic crisis was from 382.5 to 24,823.5 pg/ml (33%). **Conclusion:** The data confirm that the viruses IA and RV caused exacerbation of asthmatic crisis in adults. This virus induced the expression of HBD2 in epithelial cells, however the HBD2 expression is decreased in patients with asthmatic crisis. **Keywords:** Asthma crisis, Viral infections, influenza A (IA), Rhinovirus (RV), β-defensin-2 (HBD2).

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# *In Vitro* Evaluation of Antibacterial Activity of the Strain *Lactobacillus paracasei* KSI

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**Abstract.** The genus *Lactobacillus* belongs to a group of bacteria called lactic acid bacteria (LAB), those are named for their ability to generate lactic acid as the main or major product of carbohydrate metabolism. Some bacteria belonging to this genus are considered as probiotics; as well as compete with other microorganisms for nutrients, also they produce metabolites capable of inhibiting their growth; among them they were found low molecular weight substances with antimicrobial properties against various bacterial genera, including some pathogens. The aim of this study was to evaluate the antimicrobial activity generated by a strain of *Lactobacillus paracasei* KSI. *Methodology:* to evaluate their inhibitory effect there were performed antagonism tests by double layer method, agar well diffusion method with cell-free extract (E-A5) and minimum inhibitory concentration (MIC) by microdilution method from recovered fractions E-A5 (10X) by n-butanol, ethyl ether and ethyl acetate extractions versus *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *P. aeruginosa* 27853. *Results*: for double layer essays there were found halos of 33, 32 and 35 mm respectively were, while for agar diffusion assays testing 150 µL of cell free extract they were found 14, 14 and 0 mm, respectively. Finally in the assays for MIC there were founded values like 350, 825 and 1525 µg for *E. coli* using ethyl ether, ethyl acetate and n-butanol, respectively; besides there were obtained values like 700, 412 and 1525 µg for *P. aeruginosa* and 350, 412 and 1525 µg for *S. aureus*. *Conclusions:* an antagonistic effect against all test strains were shown, so it shows the ability of strain to generate substances with antimicrobial capacity in both liquid and solid media and offers a promising alternative to future biotechnological applications.

**Key words:** *Lactobacillus paracasei, antimicrobial activity, double layer method, agar well diffusion method, MIC*

# Analysis of transcriptional expression of f17 fimbriae and of the build of biofilm in *Gallibacterium anatis.*

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*Gallibacterium anatis* (GA) is a bacterial member of *Pasteurellaceae* family of Gram negative, gamma proteobacteria, which constitute part of the normal microbiota of poultry and other birds. There are pathogenic variants of these bacteria that are the causative agent of gallibacteriosis disease that affects oviducts. Systemic infection is also observed with this bacteria, but pathogenicity associated mechanisms remain unknown. It has been proposed the capability to build biofilm, as well as the secretion of colonization factors could be the key for this distinctive pathogenic behavior. In an attempt to know how much the development of biofilms could enable bacteria to survive and preserve its viability, in this work we measure the crystal violet dye retention of biofilms of *G. anatis* 12656-12 and SV200 strains growing in polycarbonate culture plates of 24 wells. Both strains differ in the transcriptional expression of f17 adhesin operon, as was determined measuring transcript abundances by qRT-PCR. The dye retention was quantified after 24, 48, and 72 hs post-inoculation. The cultures were growth in 2 ml of BHI broth in static chamber at 37°C. Culture broth was discarded and the biofilm was treated and quantified as described elsewhere. Viability of bacteria retained in the biofilm was also carried out after DNAse treatment in parallel assays. Results were similar: viability was diminished after 48 hs of incubation, and both, biofilm and viability rise again at 72 hs in 12656-12 strain, but biofilm was scarce in SV200 as was the transcriptional expression of f17 adhesin genes.
Results pointed to the importance of f17 genes in biofilm building, but do no explain pathogenic behavior of SV200 strain

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# Phenotypic analysis of *cdgC*::GusA-Sp$^R$ mutant of a diguanylate cyclase from *Azospirillum brasilense* sp 245

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*Azospirillum brasilense* is one of the most selected Plant Growth-Promoting Rhizobacteria (PGPR) group. The bacterium promotes the growth and development of economically important crops, using several mechanisms such as phytohormone production, nitrogen fixation, phosphate solubilization, siderophores production.

Effective colonization of plant roots by *Azospirillum* plays an important role in growth promotion. It is now common knowledge that bacteria in natural environments persist by forming biofilms. Biofilms are highly structured, surface-attached communities of cells encased in a self-produced extracellular matrix, which protects bacteria from stress conditions and enhances bacteria-plant association.

Cyclic diguanylate monophosphate (c-di-GMP) is a second messenger that regulates a variety of phenotypes, including biofilm formation, motility and virulence in multiple bacteria. The molecule is synthetized from two molecules of GTP by diguanylate cyclases (DGC) containing a GG(D/E)EF domain; while its degradation is accomplished by phosphodiesterases (PDE) with two different EAL or HD-GYP domains. (1)

The genome of *A. brasilense* Sp245 encodes for 32 predicted proteins involved in c-di-GMP metabolism(2) one of them herein named as diguanylate cyclase C, was mutated, and determined its phenotype *A. brasilense* 102C mutant obtained was impaired in swimming motility, biofilm formation, fast cellular aggregation and shown growth delay, as compared with the wild type strain.

To confirm the DgcC function, we cloned the *cdgC* gene under its own promoter in pJB3Tc20 vector, for genetic complementation, to analyze the biofilm architecture. To this end, the strains were tagged with EGFP (pMP2444 vector), and visualized with confocal microscope. The data obtained indicate that, the gene *cdgC* was able to reestablish biofilm production two-fold, as compared with WT. However, complemented strain shown higher cellular aggregation than mutant and WT strains. This latter result indicates that over-expression of DgcC displayed a contrary effect as expected suggesting that c-diGMP intracellular levels should be strictly regulated, as was observed in other bacteria.

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# Microbial diversity in the intestinal tract of the Pacific reproductive geoduck clam *Panopea globosa* (Dall 1898)

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Microbiota plays an important role in the development and health of marine organisms. As many others molluscs, bivalves present a particular biota associated to them which is classified in resident flora or established microorganisms, and in transitory biota, who refers to those bacteria that are just passing through. It is known that this unique microbiota could change from the natural habitat where the organisms live, to the culture conditions where they can be used as aquaculture broodstock. Knowledge of the bacteria associated with bivalve molluscs it is important to understand the existing balance between this microorganisms and their host. Healthy bacterial populations stimulate the host's immune system allowing it a better use of nutrients and a subsequent constant growing, which is vital for aquaculture processes. The geoduck clam *Panopea globosa* is the second most important commercial mollusc in Baja California México, and its fishery has worldwide importance. Until now, little is known about the microbiota composition associated to these marine organisms. In this work, we evaluated the diversity of cultivable bacterial present in geoduck's digestive tract of adult clams freshly recollected of their natural habitat (group 1), and from those that were acclimatized in the laboratory as breeding stock (group 2). Bacteria identification was carried out by both biochemical tests and MALDI-TOF MS analysis. A total of 130 bacterial strains were isolated, of which 111 were identified with MALDI-TOF MS methodology, 52 from group 1 and 59 from group 2. The analysis showed us that the predominant bacterial genera belong to *Vibrio*, regardless of the organisms' group. Interestingly, we observed changes in the microbiota composition of those organisms that were acclimatized in the laboratory during 8 weeks, under adequate culture conditions. In clams of the group 1 were identified *V. nereis* (48%), and *V. alginoliticus* (44%). After acclimatization of geoduck clams (group 2), we identified *V. xuii* (10.2%), and *V. gigantis* which was the predominant bacteria (57.6%) and was not identified in group 1. In both groups other *Vibrio* species, as *V. rotiferianus* were identified too.

These findings are significant due the relevance that some of the present bacteria in the digestive tract of the geoduck clam *Panopea globosa*, could has as probiotic, and be used to increase the survival and growing rate of this important fishery.

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# Isolation and characterization of influenza A virus strains in Mexican patients

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Influenza A virus causes 250,000-500,000 deaths around the world. In México, during the 2014-2015 season, 773 deaths were confirmed, 692 (89%) caused by H1N1 pdm 09. During the 2015-2016 season, 627 deaths were confirmed and 337 (54%) were caused by de H3N2 subtype. The genetic and biochemical analysis of these strains will provide us information about the antiviral resistance mutations, their relationship with other strains circulating in the population, changes of the receptors recognition, etc. The goal of this project was the isolation of influenza A virus strains in cell culture, and the analysis of the hemagglutinin (HA) and neuraminidase (HA) sequence genes. A total of 43 clinic samples (31 lethal and 10 no-lethal cases) were captured in the Epidemiological Surveillance Laboratory “La Raza” and analyzed in CIBIOR. Of lethal cases, 21 corresponded to H1N1pdm09 and 10 to H3N2, while all 13 non-lethal cases corresponded to H1N1pdm09. Several bioinformatic analysis were performed by using programs and algorithms, which allow to inferring several viral properties such as receptor specificity changes of HA and NA proteins, antiviral resistance, antigenic variation, etc. Of analyzed viruses, all HA genes from H1N1 viruses have the typical mutations 190D and 225G which confer the specificity change (sialic acid 2,6->2,3) and is involved in the low respiratory tract infection and therefore in a severe disease. The antigenic sites on the HA protein, typical to immune system escape are very similar to previous circulating strains and those viruses in the current vaccine, while 10% of NA genes presented mutations related to neuraminidase inhibitors. Due to the influenza viruses variability, a comprehensive study is necessary. Our group is working on biological and biochemical characterization of viruses presented in this abstract and isolating other viruses, to identify likely changes involved in the pathogenicity of this virus.

# "Physiological characterization of conidia of *Sclerotium cepivorum* Berk: causal agent of garlic white rot disease"

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Guanajuato is the second more important garlic producer at national level, but it is being drastically affected due to the loss of big areas of crop caused for *S. cepivorum* Berk (*Sc*B). This phytopathogen fungi, produce sclerotia (structures formed by hyphal aggregation). Sclerotia function has been reported is for reproduction and environmental resistance, that allows to the fungus stay viable in soil, until 20 years in the absence of its host, as well as the only factor of disease spreading; Leaving in second term, the capacity of this fungus to produce conidia in nutrient deficient media and high humidity. Conidia are described as sterile with no known function. One of the main factors that limit study of conidia role in the fungus biology is the reduced quantity of conidia produced and prolonged time needed to obtain them in poor medium (20 days). Our working group have obtained a mutant strain of *S. cepivorum* that lost its ability to form sclerotia, but produces conidia even in rich media. This strain could be used to explain role and process of conidiation in the fungus biology. In this work, we aimed to study physiology of conidia produced by *S. cepivorum* Berk, for which, we have identified medium and conditions needed to obtain higher number of conidia. It was observed that the mutant strain produce more conidia than the wild type strain in less time, and morphologically just there is a difference in thickness of hyphae between both strains. DAPI staining of conidia indicates that approximately 18% of them present nuclei. TEM analysis confirmed the absence of nuclei in some conidia besides allowing us to appreciate that there is no difference in the structure of conidia and that they have the necessary elements to germinate between wildtype and mutant conidia. These results allow us to consider the use of the mutant strain to evaluate the role of microconidia in the biology of this fungus. We increased the amount of conidia with nuclei by centrifugation in sucrose density gradients and these were used to evaluate germination under different germination induction conditions. The percentage of germination reached was low. Plant infection showed that conidia produced by mutant strain having ability to infect roots and seeds of garlic in laboratory experiments. We suggested that the conidia function in *Sc*B is involved in the conservation (endogenous dormancy), more than the propagation of the specie (exogenous dormancy). This work was supported by DAIP, U. de Guanajuato

**Multiple serum proteins as opsonins of *Sporothrix schenckii* conidia.**

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*Sporothrix schenckii* is a human pathogenic dimorphic fungus that causes sporotrichosis, a cutaneous subacute or chronic mycosis infection of the skin or subcutaneous tissues. Epidemiological and experimental evidences suggest that natural infection is initiated by the traumatic introduction of conidia into the skin. In infected tissue, the fungus differentiates into the yeast form and may spread to other tissues. Human tissues affected by sporotrichosis show intracellular infection of macrophages (Mϕ's), polymorphonuclear cells, and giant cells, as evidenced by budding yeast observed within these phagocytes. In the laboratory we demonstrated that optimal phagocytosis of conidia and yeast is dependent on preimmune human serum opsonisation. Monocites from human blood that were differentiated to macrophages efficiently ingested opsonised conidia. Competition with D-mannose, methyl α-D-mannopyranoside, D-fucose, and N-acetyl glucosamine blocked this process, suggesting the involvement of the mannose receptor in binding and phagocytosis of opsonised conidia. Little is known about the precise molecular mechanisms of interaction between conidia and the macrophage, but is clear that serum proteins have a role in the binding and internalization of the microorganism.

The purpose of the current study was to identify components of the human serum that opsonize conidia of *S. schenckii* favoring their phagocytosis by macrophages. We describe a phagocytosis assay with conidia opsonized by serum fractions produced by various chromatographic techniques (affinity with Con A-Sepharose and ionic exchange).

We establish that multiple serum molecules are involved in opsonisation and phagocytosis: mannoproteins that are the more efficient and proteins without mannose residue. In addition IgM mediated phagocytosis efficiently.

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# Metabolomic study of *Lasiodiplodia theobromae*

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Plant pathogenic fungi such as those from the Botryosphaeriaceae are pathogens that affect a wide variety of economically important plants, such as *Vitis vinifera* (grapevine) and *Olea europea* (olive). A particularly virulent member of this family of fungi, *Lasiodiplodia theobromae*, causes disease known as “dieback” or “black dead arm”. It has been found to infect over 500 species of plants worldwide. From a metabolomics perspective, this work identified fatty acid esters as metabolites produced by the fungus *L. theobromae* in natural substrates. This was done via the purification and chemical characterization of ethyl linoleate (LAEE), using thin layer chromatography (TLC), HPLC, mass spectrometry, nuclear magnetic resonance (NMR) and gas chromatography-mass spectrometry (GC-MS). GC-MS identified a variety of fatty acid esters. A few of these compounds showed physiological activity during germination and early growth in the plant model *Nicotiana tabacum*, inhibiting growth at high concentrations (50-200 µg/mL) and inducing germination and growth at low concentrations (25 µg/ml-98 ng/mL), with LAEE, ethyl stearate (SAEE) and ethyl palmitate (PAEE) showing significant physiological effects. Experiments on the long term effects of these compounds were done on *N. tabacum* and other plants via hydroponics, whose effects on growth depended on the plant species. In *N. tabacum*, LAEE y SAEE inhibited growth and caused chlorosis after germination and one month of growth. However, PAEE induced growth, with an effect on true leaves, which grew significantly larger with 1 µg/mL PAEE. Proteomic characterization during the production of fatty acid esters (FAE) was done using *de novo* sequencing. Many novel proteins were detected, including many pathogenicity-related proteins such as Cap 20, and cyanovirin-N, a lectin with great anti-viral activity, among many others.

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# Cloning and expression of the ORFan g26 from coliphage mEp021.

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## ABSTRACT

The genome of mEp021 bacteriophage has been sequenced and almost totally ensambled in one contig. Around a 40% of the genes are ORFans (genes of unknown function). These ORFans only present homology with hypothetical proteins (HP) or they do not present similarity with other genes in databases.

This study aims to identify the function of ORFan g26, which is located in the replication/recombination cluster of the phage genome. Firstly, *in silico* analysis was performed through NCBI and Interproscan server. We found that ORFan g26 contains 279 nucleotides and encode for a 93 a.a, protein. It shown 95% of identity with a HP of a putative prophage of *E. coli* strain UMNK88. The identity of G26 with anti-RecBCD protein of phage Sf6 of *Shigella flexneri* and P22 of *Salmonella spp.* was 34 and 32%, respectively. It also contains the PD06622 conserved domain, without assigned function. However, proteins with this domain are marked as Anti-RecBCD.

Subsequently, the ORFan g26 was amplified and cloned into the pLEX vector. It contains the $cI_{857}$ repressor gene of phage λ, whose product control the g26 expression. The expression of G26 generates a toxic effect, inhibiting the bacteria growth. High levels of protein was observed after 45 minutes of induction, therefore it is likely that during phage development. G26 is expressed and subsequently degraded or its expression is down-regulated.

To know which host protein(s) bind to G26, we are planning to use the pull-down assay. We suggest that the product of g26 ORFan could inhibit RecBCD nuclease function and therefore allow a better development of the phage.

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# Association of Human Papilloma Virus with prostate cancer in Mexican Men.

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Multiple epidemiologic studies have been demonstrated in a concluding way the existence of a great diversity of infectious agents that cause cancer around the world. Between these agents, the Human Papilloma Virus (HPV) represents the most common sexual transmission disease and the main risk factor to development cervical cancer and anogenital tumors. The association between HPV and the development of prostate cancer (PCa) has been suggested; also inflammation of the prostatic tissue secondary to sexual transmitted infections may participate during the course of carcinogenesis. In this study, we investigated the association between the HPV presences with the development of PCa, We performed a case control study in which we analyzed 356 samples of prostatic tissue; 189 samples were diagnosed with prostatic adenocarcinoma and 167 samples with Benign Prostatic Hyperplasia (BPH). The presence of HPV was identified by PCR technique using universal oligonucleotides (L1, MY and GP) and a genotyping process by using multiplex PCR technique. We identified 8 different viral genotypes, classified as oncogenic and non oncogenic genotypes (6, 11, 16, 18, 31, 33, 52 and 58) in 37 and 16 PCa and BPH samples respectively. A high prevalence for the HPV 52 oncogenic genotype was found in the PCa and BPH samples, while in the multiinfected samples of PCa, a high prevalence of HPV genotypes 16 and 18 were detected. Finally in BPH the genotypes were HPV 11, 52 and 58. The results showed an increased risk of developing PCa in the presence of HPV infection with an OR = 2.2973, IC = 95% = 1.2257 – 4.3057. We concluded that the HPV infections can contribute to the risk of developing PCa.

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# Searching for cell envelope components involved in the coliphage mEp021 infection.

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A crucial initial step for any phage infection is the recognition and interaction with receptors of the cell envelope. In this study, we used the mEp021 to know which cell envelope factors are required for infection. mEp021 belongs to a new “non-lambdoid” phage group. The common property of mEp021 and lambda is that both require Nus host factors to grow.

mEp021 infection was tested in 47 different cell envelope mutants of *Escherichia coli* K-12, which were obtained from the Keio collection. A partial inhibition of the viral particles production, up to 2 logs, was observed in *srl*B$^-$ and *liv*G$^-$ mutants. The product of these genes are involved in an ABC transporter and in the IIA component of the phosphotransferase system glucitol/sorbitol, respectively. Specific primers for these genes were designed and a PCR assay was performed to verify the identity of these mutants. The presence of the corresponding amplicon for each gene was observed in the strain *E. coli* wild type, but not in the mutants.

These genes will be cloned in a plasmid where we expect that complementation assay restore its function and the mEp021 infectivity.

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# Influenza A virus downregulates the expression of thrombomodulin in endothelial cells

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Introduction: Influenza viruses are one of the main pathogens that affect humans and have the ability to generate worldwide pandemics. Infection with these viruses can cause severe clinical symptoms causing the loss of many human lives, as happened in the 2009 pandemic (H1N1). Much of the deaths may be associated with a heightened inflammation, manifested as increased proinflammatory cytokines, and an imbalance in the process of vascular thrombosis. The vascular endothelium is the tissue responsible for regulating hemostasis through proteins expressed on its surface, such as Thrombomodulin (TM). The TM is associated with the regulation of coagulation and inflammation. One function of TM is to activate protein C by forming a complex with thrombin and once protein C is activated, it inactivates by proteolysis the V and VIII factors of the coagulation cascade, thereby inhibiting the formation of thrombi. The hypothesis arising from this background; is that the influenza virus decreases TM levels of the endothelium thus promoting systemic thrombosis.

Objective: The purpose of this work is to evaluate whether the infection of HMEC-1 endothelial cells with influenza AH1N1 virus modifies the expression of TM.

Materials and Methods: HMEC-1 cells were infected with influenza A H1N1 virus-pdm09 at a multiplicity of infection of one. Tests were performed by western blot, from total protein extracts obtained at 1, 6 and 24 hours. To evaluate gene expression total RNA was extracted using TRIZOL in infected cells under the same conditions, then cDNA was synthesized and real time PCR were performed with specific primers for TM and the viral protein M1. TNF-α was used as a positive control of the TM expression at 10 ng/mL and retinoic acid was used a negative control at 1 uM.

Results: Similarly to what is already reported the stimulation of TNF-α in HMEC-1 cells, so the influenza virus is capable to promote a decreased expression of the TM protein level at six hours post infection, this effect remains until 24 hours. This same lowering effect could be detected at the level of gene expression at the same times of infection. Furthermore, this observed effect can be associated with an increased viral gene expression.

Conclusion: Endothelial cell infection by influenza A virus affects thrombomodulin expression at the transcriptional level. The downregulation of thrombomodulin promoted by the virus could favor deregulation of coagulation and inflammation processes.

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# Cloning and expression of genes encoding fimbrial proteins F17 of Flf3 operon from *Gallibacterium anatis*.

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ABSTRACT

Introduction: The bacteria *Gallibacterium anatis* is considered a clinically important bacterial agent, its importance is in the veterinary and economic fields, this microorganism is responsible for salpingitis and peritonitis in egg-laying chickens, affecting the egg industrial production by 40%. Due to the clinical and economic importance, it is important to investigate in the genetic level the components involved in the expression of fimbrial proteins F17 in *Gallibacterium anatis*, as a crucial element of the mechanisms of pathogenicity of this bacteria. Objectives: Cloning in *E. coli* fimbrial gene of Flf3 operon from *Gallibacterium anatis*, encoding the structural and adhesin proteins F17. Subcloning fimbrial proteins in pQE30 expression vector and inducing protein expression. Material and Methods: the cloning protocol of the fragments of interest was done in the cloning vector pBluescript KS (-). Clones were obtained in *E. coli* strain DH5α. The clones were verified by PCR, digestion with HindIII and KpnI enzymes and sequencing. The gene were subcloned in the expression vector pQE30. The two constructs containing subcloned fragments corresponding to structural and adhesine proteins were induced with IPTG. Samples were analyzed on acrylamide gels by SDS-PAGE. Results: Two transformants were obtained in *E. coli* of fimbrial operon Flf3 from *Gallibacterium anatis* strain ESV34, these were verified by PCR and digestion, one clone of 3463pb, corresponding to the structural protein gene, the insert size was 493pb and the other construction of 3715pb, was a gene encoding a adhesine protein, the insert size is 745pb. The subcloned fragments were obtained in the expression vector pQE30, the transformants were induced with IPTG. The protein expressed size was 18 kDa for the structural protein and 27.6 kDa for the adhesine protein. Discussion: The transformants obtained from recombinant proteins of Flf3 operon from *G. anatis*, are likely candidates to be expressed as antigens and induce an immune response in poultry, so it could be used as an alternative strategy for vaccine development.

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# Conservation of cell polarity markers in the Kingdom Fungi

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Cellular polarization is a shared feature of most fungi, conspicuously evident during budding in yeasts and hyphal growth in filamentous fungi. Polarized growth requires the concerted action of cytoskeletal components, multi-protein complexes (polarisome and exocyst) and cell-end markers that support the transport and site-specific delivery of secretory vesicles to the growth pole. Those vesicles provide lipids for expansion of the plasma membrane, as well as the enzymatic machinery that synthesizes and remodels the cell wall. The increasing number of available fungal genomes has allowed us to mine the genomes of representative species within each fungal phylum and analyze the molecular diversity of their putative cell polarization machinery. We will show the conservation of well-recognized cell polarity markers in the Fungi, along with an individualized analysis of their molecular divergence aimed to explain the observed differences of the fungal polarization machinery at the cellular level.

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# IL28B gene polymorphisms rs8099917 and rs12979860 with response to treatment with Pegylated Interferon and Ribavirin in patients with Hepatitis C

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It has been shown that the presence of polymorphisms single nucleotide (SNP) rs12979860 and rs8099917 linked to the gen of Interleukin 28B (IL28B) is associated with an increased chance of spontaneous healing and improved response rate to antiviral therapy with pegylated interferon and ribavirin in patients with HCV infection.

The data related to this polymorphism in the Mexican population are scarce. This study aimed to determine whether there is an association of IL28B gene polymorphisms with response to treatment with pegylated interferon and ribavirin in hepatitis C infected population in the Hospital "Dr. Aquiles Calles Ramirez" of ISSSTE.

57 patients diagnosed with hepatitis C, with treatment completed with pegylated interferon and ribavirina were studied from January to December 2015 .

For genotyping IL28B rs12979860 polymorphism, allelic discrimination assay with SNP genotyping kit with predesigned Taqman probes by Applied Biosystems was used. The tetra-primer amplification refractory mutation system it was used for determining the rs8099917 polymorphism.

Regarding to genotype rs8099917 in the study population, the heterozygote TG predominated with 54%, followed by the TT homozygote with 30% and finally the GG (16%).

The allelic frequency was T = 57% and G = 43%. In the case of rs12979860 predominates in this population the heterozygote TC 34 (60%), followed by the TT homozygote with 13 (23%) and finally the CC 10 (18%). In both polymorphisms was fulfilled with Hardy-Weinberg Equilibrium and none of the three main models inheritance (dominant, co-dominant and recessive) the statistical data support the association between genotypes and response to treatment.

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# Disruption of anion exchanger 1 (band-3) by plasmid-encoded toxin a serine protease from enteroaggregative *Escherichia coli.*

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Enteroaggregative *Escherichia coli* infect the human intestine and produce diarrhea. The clinical features of the patients are associated with the synthesis of plasmid-encoded toxin (Pet) by the bacteria. Pet toxin is a member of the autotransporter (type V) protein family, included in the serine protease autotransporters of the *Enterobacteriaceae* (SPATEs) subgroup. In this work we report that this protease alters the anion exchanger 1 (AE1) or band 3 protein, a membrane integral protein, 95- to 100-kDa, and that is part of the SLC4 family; ion exchangers chlorine and bicarbonate. Pet protein was obtained from a culture supernatant of *pet* clone *E. coli* HB101(pCEFN1). For AE1 (band-3) protein degradation assay, membranes from sheep red blood cells (SRBC) and rabbit small intestinal and colon mucosa proteins was incubated with the toxin. Pet induce AE1 (band-3) protein degradation and produces a 70-kDa subproduct and it was identified using AE1 (band-3) antibodies. This event was inhibited when it is incubated with a mutated version of Pet (S260I), with the inhibitor of serine protease (PMSF) and with neutralizing antibodies anti-Pet. In samples of intestine and colon antibodies anti-AE1 (band-3) showed an immune cross-reactivity to a polypeptide dimeric of 50-kDa that was susceptible to degradation by Pet. The results show that AE1 (band 3) is a molecule sensitive to Pet, where the structure of the cell membrane and ion exchange can be altered.

# TEA proteins in apical organization in *Neurospora crassa.*

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Microtubule associated proteins (MAPs) is a heterogeneous group of motor and non-motor proteins. They are involved in the regulation of the MTs dynamics in different cellular processes, for example, dynamic instability that promotes the stability, or polymerization and depolymerization of MTs. In *Schizossacharomyces pombe* a gropu of MAPs call TEA has been described. There are three members of this group: Tea1 (Tip Elongation Aberrant protein) that is actively transported in the plus end of the MTs and accumulates in places wherepolarized growth takes place. Close to the plasma membrane, Tea1 is associated with Tea4 and Mod5 to finally modulate the function of a formin, that is responsible of actin filaments polymerization. .

In this study, we looked for the othologues of Tea1, Tea4 and Mod5 in the filamentous fungus *Neurospora crassa* and named them TEA-1, TEA-4 and TEA-5. We produced the deletion mutants of the *tea-1* and *tea-4* genes and functionally characterized them. We observed that both mutants Δ*tea*-1 and Δ*tea*-4 had an slight decresed in growth rate -1.3% and 10% respectively. This difference was higher when we measured biomass production per day, 18% in Δ*tea*-1 mutant and 10% in Δ*tea*-4 mutant, relative to the wild type strain. Hyphal morphology is not affected in both mutants, although, hyphal diameter seems to be bigger in the Δ*tea*-4 mutant. Branching is not affected in the Δ*tea*-1 mutant but Δ*tea*-4 mutant produced a higher number of branches than the wild type. Conidiation was affected in both mutants Δ*tea*-1 and Δ*tea*-4, we observed a reduction of 80.3% and 81.2% respectively compared with the wild type strain.

Evidence suggests that this group of proteins seems to be not involved in the directionality of polarized growth, but are involved in the selection of the site where the polarity will begin, however, the necessary analysis to prove are still ongoing.

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# Zip14 role in regulating the redistribution of plasma zinc into liver during inflammation and their induction by signaling pathway Jak-Stat

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After trauma, infection and surgeries are released pro-inflammatory cytokines which initiate an acute phase response. The use of an experimental surgery in rats as model of inflammation has shown the existence a decrease of zinc in plasma, and the increase of this metal to liver, however the mechanism involved in this redistribution is not completely known. Therefore the aim of this work was study the role of Zip14 in the hepatic uptake of zinc at the expense of serum zinc, as well as signaling pathways involved in the expression of Zip14 after an abdominal surgery. Thirty-five male Wistar rats were subjected to experimental surgical stress, then; subgroups of five animals each were sacrificed at 3, 6, 9, 12, 16, 20 and 24h. Matched groups without surgery were used as controls. Zinc levels were determined by AAS, and the intracellular zinc with both zinquin and dithizone staining. Hepatic metalothionein was assayed by a Cd-saturation method, and IL-1B, IL-6, and TNF-a by immunoassays. Zip14 expression was analyzed by RT-PCR real-time, and protein level by immunohistochemistry and Western-blot. Experimental surgery produced a hypozincemia, and the increase of zinc in liver, also produced the release of IL-1b, IL-6 in serum, and the increase of hepatic MT. Histochemistry showed a decrease of intracellular free zinc at 3 and 6 h, but an increase at 9h (zinquin), meanwhile, total intracellular zinc increased after 9h (dithizone). The RNAm and protein level of Zip14 were elevated between 6 and 20h after surgery. In the rats administered with a Jak2 inhibitor, the level of Zip14-mRNA and its protein level were decreased after 12 h of surgery; same pattern was showed after the administration of a Stat3 inhibitor. Inhibition of NFkB increased the level of Zip14 mRNA, however the protein level did not show changes. On the other hand, inhibition of JNK and MAPp38 did not produced changes in the Zip 14 mRNA, but the protein was decreased. These results suggest that during a surgical trauma, Zip14 is one of the major transporters involved in the zinc redistribution from plasma into liver and its expression is dependent of the signaling pathway Stat3 Jak2-mediated by IL-6.

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# Effect of testosterone on oxidative stress in blood and spleen of CBA/Ca mice infected with *P. berghei* ANKA.

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Malaria is a disease caused by the parasite *Plasmodium.* According with the WHO, malaria was responsible of 485, 000 deaths in 2014. Malaria displays sexual dimorphism in which males develop higher mortality and symptoms that females. Males have higher concentrations of testosterone whereas females possess higher levels of oestradiol. Nevertheless, the effect of the sexual hormones during *Plasmodium* infection has been poorly studied. Oxidative stress is the main mechanism to eliminate parasites by the immune system. Macrophages produce oxidizing species such as nitric oxide, superoxide radical peroxide, hydroxyl radical and hydrogen to oxidize the parasite membrane. However, the oxidant species don't discriminate between the parasite and the host. To deal with the negative effects of oxidative stress, the host produces three main enzymes: the superoxide dismutase (SOD), the glutathione peroxidase (GPx) and the catalase (CAT). Increasing their activity reduces oxidative stress and could protect *Plasmodium*. The aim of this study was to study the role of testosterone on oxidative stress and antioxidant response in *P. berghei* ANKA infected mice. Groups of CBA/Ca mice were gonadectomized, after a month of recovery, were reconstituted with testosterone for 3 weeks, and were infected with *Plasmodium berghei* ANKA. These mice and their controls were sacrificed on day 8 post infection, blood and spleen were extracted, to quantify the activity of SOD, CAT and GPx. In addition, we also evaluated the MDA concentration. Our results show that gonadectomy reduced SOD and GPx activities in both infected and non-infected mice, which in turn increased the MDA levels in blood. Treatment with testosterone decreased GPx activity in both male and female infected mice, but did not affect the GPx activity in infected gonadectomized mice. In the spleen, gonadectomy decresed SOD activity in both tissues blood and spleen as a result MDA levels increased. In addition, administration of testosterone to intact mice decreased catalase activity but did not show effect on gonadectomised mice which in turns increased the MDA levels in this group. We conclude that gonads are involved in the regulation of antioxidant activity, probably because they produce sex hormones, that in turns regulate the expression of antioxidant enzymes. This work was funded by DGAPA, PAPIIT IN216914

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# Purification of $IgG_1$ and $IgG_2$ from hyperimmunized bovine against *Anaplasma marginale*

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Bovine anaplasmosis is an important infectious disease distributed worldwide. It is caused by the rickettsia *Anaplasma marginale*. The disease can be fatal in adult cattle and it is characterized by fever, weight loss, anemia, decreased milk production and abortion. *Anaplasma marginale* is an obligate intracellular pathogen transmitted by hematophagous arthropods or via contaminated surgical instruments.

Tetracycline is tipically used to treat acute infections, however rickettsia is not eliminated and infected cattle become carriers. In Mexico there are no commercial vaccines available against *A. marginale* infection; furthermore this pathogen makes use of several mechanisms of immune evasion. On the one hand, diverse immunogens have been evaluated, but these have been shown to induce only partial protection against heterologous challenge. On the other hand, protective immunity against anaplasmosis requires production of immunoglobulin $G_2$ ($IgG_2$), which enhances phagocytosis and production of nitric oxide by activated macrophages.

In this study, initial bodies isolated from infected blood with mexican *Anaplasma marginale* strains were inoculated to cattle via subcutaneous injection. We used saponin-based *adjuvant* to increase the immune response. We collected serum samples for monitoring antibody production by serological assays. When antibody levels were high, animals were challenge-inoculated with infected red blood cells. $IgG_1$ and $IgG_2$ were purified from immunized bovine serum using dialysis and ion-exchange chromatography. Their purity was monitored by immunodetection using anti-heavy chain specific antibodies. This molecules could be useful for designing diagnostic assays or for understanding host-pathogen interactions.

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# Analysis of differential expression between strains of *Trypanosoma cruzi*, with high or low infectivity

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The parasite *Trypanosoma cruzi* is the etiologic agent of Chagas disease. This parasite has a complex life cycle that involves hematophagous triatomine insects as vectors for transmission, and a broad range of mammalian host that include humans, domestic animals and sylvatic as reservoirs. Although it is known that the biological features of *T. cruzi* are determining in the pathogenesis of Chagas disease, little is known about the factors that determine the infectivity and virulence of the parasite. Therefore, in this work, we propose the study of molecules that could be involved in the infectivity of *T. cruzi*.

In a previous study of RNAseq and proteomic analysis, we identified transcripts and protein sequences, that are differentially expressed between the parasites clones, with high (NM1cl1) and low (CI2cl2) infectivity. From these data, in this work we analyzed the previous results, finding that 259 and 87 sequences that are overexpressed bothin transcripts and proteins in NM1cl1 clone and CI2cl2 clone, respectively. Besides, these sequences were over-express in a fold change from 1.24 to 36.30 in transcripts and from 1.0 to 22.75 in proteins. Using this data, we selected a sequence with the highest fold change (22.75) in both transcripts and proteins, that is annotated as a hypothetical protein in the TriTrypDB database of *T. cruzi*. The alignment analysis of the selected sequence, indicated that this molecule could be grouped into the complement regulatory proteins (CRP) of the parasite. Initial functional analysis of the molecule under study, will be presented.

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# Clustering of Ig-like domains during CRTAM-NECL2 Interaction

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Cell adhesion molecules (CAMs) are essential for the maintenance, effector functions of the immune system and cell-cell communication. These are mediated by interactions of receptor-ligand homophilic type and heterophilic, which is to be shown that the formation of groups ("clusters") play an important role in increasing the avidity interaction and maintain the architecture (Mackat and Imhof , 1993; Issekutz, 1993). CRTAM is a transmembrane protein belonging to the immunoglobulin superfamily and its ligand Nectin-Like 2 that is expressed on dendritic cell surface (DCs), epithelial cells and neuronal synapse. However, it has not been characterized function proximal to the transmembrane region domains.

The results show that the constant domain CRTAM present oligomerization in solution, this in turn suggests that the interaction of CRTAM and Nectin-like 2 with the constant domains present in both molecules could be implying increased avidity, obtaining a better affinity Kd = $3.72x10^{-8}$ M.

In the same project also it aims to characterize the epitope of the monoclonal anti-CRTAM previously made by the working group (Patino-Lopez, 2006). Seizing CRTAM constructions was achieved by immunoblot find that the domain Ig2 of CRTAM is the antigenic determinant and the monoclonal anti-crtam has a Kd = $6.28x10^{-10}$M.

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# Effect of oestradiol on the mRNA expression of IFN-γ and TNF-α in CBA/Ca mice infected with *P. berghei* ANKA.

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**Introduction:** Malaria is a infectious disease caused by parasites of the genus *Plasmodium*, which in 2015 produced 438 000 deaths. Sexual dimorphism is a feature of this disease, males develop higher mortality than females. Sex hormones are responsible of the main differences among sexes; therefore, it is probable that sexual steroids be involved in the immune response against *Plasmodium.*. In addition, malaria induces a strong inflammatory response mediated by cytokines such as TNF-α and INF-γ which are important to eliminate the parasite.

**Hypothesis:** Oestradiol ~~may~~ modulates the ~~+~~mRNA expression of TNF-α and INF-γ in a malaria infection.

**Objectives:** To assess the effect of oestradiol on the mRNA expression of TNF-α and INF-γ in CBA/Ca mice infected with *P. berghei* ANKA.

**Methods:** Groups of mice were treated with: oestradiol or with the vehicle Half of these groups were ~~–~~infected with *P. berghei* ANKA In addition, groups of gonadectomized (Gx) mice were treated with ~~t~~ oestradiol and infected with the parasite. Parasitaemia was evaluated daily, and on day 8 post infection mice were sacrificed to isolate RNA from blood, spleen, liver and brain. The RNA was reverse transcripted and the cDNA was used to evaluate the expression of IFN-γ and TNF-α by real time PCR.

**Results and discussion:** Gonadectomy increased parasitaemia in female mice. In contrast, parasitaemia decreased in male mice. Reconstitution of Gx female mice with oestradiol decreased parasitaemia compared with control mice that received vehicle. These results show the importance of oestradiol in the control of parasitaemia.

Infected mice treated with oestradiol increased the liver mRNA expression of both IFN-γ and TNF-α. In contrast, Gx mice reconstituted with oestradiol decreased the expression of IFN-γ and TNF-α. This finding suggest that oestradiol regulates the expression of both IFN-γ and TNF-α depending on the concentration.

Females had higher TNF-α expression that the males in blood, spleen and liver tissues. Meanwhile males had a higher expression of TNF-α in brain that the females, this could explain the reason why males are more susceptible to complications such as cerebral malaria.

**Conclusions:** A clear sexual dimorphism in TNF α expression was observed in brain.

oestradiol modulates the expression of proinflammatory cytokines IFN-γ and TNF-α.

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# *Trypanosoma cruzi* RNA polymerase I: Characterization of the nuclear localization signal of subunit RPA31

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Trypanosoma cruzi is a member of the protist kingdom and so a unicellular eukaryotic organism. As part of the phylum Euglenozoa *T. cruzi* is classified within the family Trypanosomatidae. This microorganism is of medical importance as it is the causative agent of Chagas disease, and it is also an interesting biological model because of its atypical structures in its cellular composition. The RPA31 protein is a subunit of RNA polymerase I. It known to be an essential and specific protein in Trypanosomatidae family members. In this work it was possible to clone the ortholog gene TcRPA31 of *T. cruzi* and generate recombinant proteins fused to the coding sequence of EGFP fluorescent protein. It was found that trypanosomes transfected with these genetic chimeras show fluorescent signal in the nucleolus. With subsequent computer analysis we were able to identify to the carboxyl terminus a deca-peptidic sequence Pro, Ile, Arg, Lys, Thr, Arg, Ala, Lys, Lys, Glu as a potential nuclear localization signal. We have determined that chimeric constructs in which this signal has been removed completely import potential core have a cytoplasmic localization. We will present the subcellular localization of additional mutant versions of this specific nuclear localization signal.

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# *Characterization of the* cyst *formation in vitro of Toxoplasma gondii* and study of the role of the cytoskeleton of the host cell

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BACKGROUND: *Toxoplasma gondii* presents 3 infectious stages; sporozoite, located within oocysts, tachyzoite highly motile, invasive and replicative form and bradyzoite, a slowly replicative form contained within tissue cysts. The tissue cyst is a structure surrounded by a cyst wall, which protects parasites from cells and molecules of the immune response as well as from parasiticidal drugs. Parasite encystment in the host is triggered by certain molecules of the immune response such as IFN-γ. *In vitro*, cyst formation can be induced by growing parasites in infected cells under alkaline pH, thermal shock, nutritional stress or by the presence of cytokines. In our laboratory, we induced encystment of *T. gondii in vitro* by exposure of tachyzoites to an immunosuppressive drug that is an inhibitor of the synthesis of DNA and RNA. The aim of this study is to characterize in detail the biogenesis of the cyst and analyze the changes occurring in the host cell cytoskeleton during such parasite differentiation *in vitro*.
METHODS: HEp-2 cells were infected with tachyzoites previously treated with the cystogenesis inducer at different times and concentrations. Using confocal/electron microscopy we evaluated rearrangements of cytoskeletal proteins of the host cell as well as the expression of the CST1 glycoprotein of the cyst wall and GAP45 protein specific of the parasite membrane in order to see the reorganization of the cytoskeleton parasite during differentiation. Actin filaments, microtubules and intermediate filaments are the components of the cytoskeleton, which will be evaluated by using specific markers.
RESULTS: Our results showed that inhibition of DNA and RNA synthesis induced encystment in vitro without affecting host cell viability. *Toxoplasma* encystment induced rearrangements of the components of the cytoskeleton that correlated with modifications the cell shape to finally reach a cyst structure.
CONCLUSIONS: The exposure to this inhibitor induced tachyzoites differentiation to bradyzoite in a process that is dependent of the host cell cytoskeleton

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# ERM proteins of mosquito *Aedes* sp., important vector of human disease.

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Dengue, chikungunya and zika viral infections have high incidence around the world, mainly in the tropics and subtropics and nowadays, about 2.5 billion people are at risk of infection. These pathogens are transmitted through the bite of female *Aedes sp*., mosquitoes and the more efficient control of these diseases is through the vectors combat. Traditional strategies employed for the control of mosquito disease vectors, such as insecticide applications, are compromised due to the rapid development of resistance by the vectors and high cost and contamination danger of these chemicals. Therefore, there is a need to understand the biology of the mosquito vector, in stress conditions including xenobiotic chemicals, as insecticides, presence and viral infections, in order to propose novel strategies to intervene transmission. The ERM proteins (ezrin, radixin and moesin) and Merlin (Moesin-Ezrin-Radixin-like protein) (ERM/M) form a highly conserved branch of the FERM (band four-point-one, ezrin, radixin, moesin) domain family. Important insights into ERM function had come from model organisms, as the fruit fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans*. ERM proteins play important roles in signal transduction, adhesion and motility of the cells, mainly regulating actin filaments remodeling. ERM proteins are major components of the cell cortex, and they cross-link plasma membrane phospholipids and proteins to the underlying cortical actin cytoskeleton. They are concentrated at cell-surface structures such as microvilli, filopodia, uropods, ruffling membranes, retraction fibers, and adhesion sites, where actin filaments associate with the plasma membrane. There are evidence that ERM proteins could modulate hepatitis C and influenza virus entry and development, and parvovirus-induced secretory route modification.
In this work, the genes of Moesin and Merlin orthologs were identified in *Ae. aegypti* derived databases and domains were analyzed by *in silico* analysis. The domains of these ERM proteins, the globular N-terminal FERM domain, which interacts direct or indirectly with transmembrane proteins, the central-helical region and the hydrophilic C-terminal tail that binds to F-actin or form a close structure through its own FERM domains were also identified in Moesin; the F-actin domain of Merlin was identified at N-terminal end, as observed in mammals and *Drosophila*. The expression of these proteins during life cycle, and in isolated organs of *Ae. aegypti* adult females and C6/36 HT and Aag2 *Aedes* sp., model cells were analyzed by WB using heterologous anti- Moesin and -Merlin antibodies. We will analyze the behavior of ERM/M proteins in *Aedes sp.* mosquitoes during viral and xenobiotics challenges and the effect of specific inhibitors of their function to understand their roles during stress conditions.

**References**: Trofatter et al., Cell 1993, 72:791-800; Bretscher et al., Nat Rev Mol Cell Biol 2002, 3:586-599; Fehon et al., Nat Rev Mol Cell Biol 2010, 11:276-287; Chen et al., J Mol Biol 2014, 426:3118-3133; Cázares-Raga et al., 2014, J Proteomics 111:100-112; González-Calixto et al., J Proteomics 2015,119:45-60.

# Effect of tamoxifen on the oxidative stress in CBA/Ca mice infected with *Plasmodium berghei* ANKA.

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**Introduction**. Malaria is the infectious disease responsible of the higher mortality in the world, it is caused by *Plasmodium*. It is characterized by sexual dimorphism manifested for a higher mortality in males compared with females. Since sex hormones are responsible for the main differences among the sexes, in this study we investigated the role of estradiol on oxidative stress (which is the main way to eliminate the parasite) in an experimental model of cerebral malaria.

**Methods**. In order to study the oestradiol effect on oxidative stress in malaria, we *in vivo* blocked the oestrogen receptor using tamoxifen, afterwards we infected CBA/Ca mice with *P. berghei* ANKA and evaluated the activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx). In addition, we evaluated both the nitric oxide and MDA concentration in serum, spleen, liver and brain.

**Results**. Tamoxifen treatment in infected mice increased SOD activity in the liver; increased GPx activity but only in brain. As a result it decreased MDA concentration in liver.

**Conclusions**. To block the estradiol receptor with tamoxifen did not change both the activity of the enzymes SOD and GPx and the concentrations of nitric oxide or MDA in infected mice with *P. berghei* ANKA. These findings suggest that oestradiol does not affect oxidative estress in the experimental model used.

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# Parasitological study of streams and channels of the Rancheria La Cruz de Olcuatitán Nacajuca, Tabasco

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SUMMARY
In this work a study was performed to detect the presence of parasites of public health importance, in streams and canals Rancheria La Cruz de Olcuatitán, Nacajuca, Tabasco as this contaminated water harms the population of marine species that inhabit these streams and channels as: hicotea, guao and pochitoque, among others because these spices are of human consumption.
In this study samples were taken water for 3 months in different parts of the creeks and channels. These were analyzed directly which were observed parasitic forms. Fertile eggs of Ascaris lumbricoides sp, Balantidium coli sp, Trichuris trichuria eggs.
Trichuris trichuria: A nematode Trichuris trichiura. It is a common infection that mainly affects children. Children may become infected if they swallow soil contaminated with whipworm eggs. When the eggs hatch inside the body, the whipworm sticks inside the wall of the large intestine, symptoms range from mild to severe. Symptoms range from mild to severe. Sometimes, there are no symptoms. A serious infection can cause, bloody diarrhea, iron deficiency anemia, fecal incontinence (during sleep), rectal prolapse (rectum is ejected from the anus).
Ascaris lumbricoides: Ascariasis is caused by ingestion of infective eggs. No symptoms, in severe cases, abdominal cramps and, occasionally, a grouping of nematodes block the intestines, Symptomatic ascariasis is rarely diagnosed on clinical grounds alone because the pneumonitis, eosinophilia, and intestinal symptoms are similar to those caused by other infectious agents. Infections before the appearance of eggs in the feces, infections with only male worms, and extra intestinal infections are difficult to diagnose. Adult worms can also block other parts of the digestive tract as the appendix, bile duct and duodenum, which produces similar symptoms.
The importance of the study was to detect the parasites that contaminate these waters, as they can be the main vehicle for disease transmission. So it is necessary that health authorities take measures to prevent humans and animals make contact with these contaminated waters and dangerous to health.

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# *"Identification and characterization of a phosphatase 2C of Toxoplasma gondii and its possible role in regulation of conoid extrusión"*

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**Introduction**: *Toxoplasma gondii* is an obligate intracellular parasite that infects all warm-blooded animals causing toxoplasmosis with coreorretinitis, damage to the central nervous system, lungs, heart, abortion and death. Invasion by T. gondii has been extensively studied, but signals that activate such process are unknown. It is known that there are protein kinases that are actively involved in the processes of motility, conoid extrusion and cell invasion. However, little is known about protein phosphatases in these dynamic processes. We found a PP2C protein in the cytoskeleton fraction of the parasite and we evaluated its possible role in invasion and conoid extrusion. **Methods**: We obtained a polyclonal antibody against a synthetic peptide of the PP2C of *T. gondii* designed by bioinfomatic analysis. The specific inhibitor sanguinarine against PP2C was used. **Results**: Western blot analysis showed that PP2C of about 47 kDa is mostly distributed in the cytoskeleton of the parasite. Distribution of PP2C by confocal microscopy was confined to the apical end in extracellular tachyzoites at resting state as well as during conoid extrusión, during invasión and intracelular proliferation. PP2C colocalized at the conoid zone with an apical kinase previously studied in the laboratory. Moreover, participation of PP2C in conoid extrusion was determined by the using sanguinarine, a specific inhibitor for phosphatase. Exposure affected conoid retraction remaining parasites permanently with the conoid projected. **Conclusion**: A PP2C is identified in Toxoplasma with a permanent localization at the apical end, the treatment with sanguinarine had a remarkable effect on the conoid extrusion process. Therefore, PP2C appears to be a regulatory enzyme for conoid extrusion process.

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# Effectiveness of both plants: *Cymbopogon citratus* and *Curcuma longa* as prophylactic antimalarial treatments in *Plasmodium berghei* ANKA infected CBA/Ca mice.

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## Background

Malaria is a protozoan infection responsible of 214 million cases and 438 thousand deaths in 2015. Immune response to *Plasmodium* infection is characterized by the production of pro-inflammatory cytokines and cellular immune responses that lead to inflammation. Following this protective response, regulation of inflammation it is important to prevent its adverse effects. The infection induces weight loss, fever and anemia. Some plants such as *C. longa* and *C. citratus* have both anti-inflammatory and antimalarial properties. However, these plants have never been tested as prophylactic treatments. In this work, we assessed the prophylactic antimalarial activity of both plants.

## Aim

To evaluate the prophylactic antimalarial effect of *C. longa* and *C. citratus* in mice infected with *P. beghei* ANKA

## Methods

We use 4 CBA/Ca mice groups administrated twice (-4 day and zero day) with either *C. longa, C. citratus,* chloroquine or vehicle. We daily evaluated parasitemia, weight loss, temperature, hemoglobin levels and survival.

## Results

The groups of mice treated either with *C. citratus* or chloroquine developed similar survival which was statistically higher than the groups treated with either the *C. longa* or vehicle.

The groups of mice treated with either *C. citratus* or chloroquine developed similar parasitaemias during the infection. *C. longa* treated mice had the same antimalarial effect until day 10 post infection, thereafter the parasitemia had a significant increase.

Mice treated with *C. citratus* regulated more effectively temperature and weight loss than the group of mice treated with chloroquine. However, anemia was similar in the groups treated with *C. longa* and *C. citratus*.

## Conclusions

We showed the prophylactic antimalarial effect of *C. citratus* and *C. longa* treatments. In particular *C. citratus* which elicited higher survival and a lower parasitemia compared with *C. longa*

This work was supported by DGPA, UNAM, PAPIIT IN216914.

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# Development of a detection method of *Acanthamoeba castellanii* with gold nanoparticles

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Introduction: *Acanthamoeba castellanii* (*Ac*) is a free-living amoeba that is the causative agent of diverse infections such as amoebic keratitis and granulomatous amoebic encephalitis. In recent years, it has increased worldwide the number of reports of infections by this parasite. A quick diagnosis of *A. castellanii* would help to provide adequate and timely treatment to patients, avoiding the progression of the disease and thus the subsequent death of the patient. Currently, there is not rapid diagnosis and effective method for determining the presence of *A. castellanii* not only in clinical samples but also in environmental samples.

Objective: To analyze the gold nanoparticle- antibody complex recognition of the amoeba in order to obtain a diagnostic method.

Methodology: Polyclonal antibodies which were produced in New Zeeland rabbits by immunization with total homogenates with (Ab *Ac*+i) and without (Ab *Ac*–i) interaction with the host cell (ATCC CCL -1 L929) under a classical immunization protocol by the method of Zola (1990) were obtained. Quantification of the antibody was performed by ELISA and we also perform immunolocalization of the antigenic proteins of *A. castellanii* with anti -rabbit IgG antibodies coupled to FITC. The samples were visualized on an epifluorescence microscope.

We worked synthesizing gold nanoparticles by Turkevich' method and coating its with cysteamine for coupling antibodies and we used this complex to perform the detection of antibodies by ELISA.

Results: Polyclonal antibodies against *Ac* were obtained, those who interacted with the host cell showed recognition in a range of dilutions from 1: 150 to 1: 2500, in contrast, antibodies obtained from *Ac* without interacting recognize lower titers in the range of 1: 150 to < 1: 1250. The location of the antigenic proteins in *Ac* was in cytoplasm and plasma membrane mainly. The coupling of the nanoparticles to antibodies (Ab *Ac*–i) showed increased antigen recognition in a range of 1 : 150 to 1 : 3200 for *Ac* condition without interacting with the host cell. At the present time coupling nanoparticle – antibody (Ab *Ac*+i) is performed.

Conclusions: the Gold Nanoparticle- Antibody complex showed an increased recognition of amoeba at low concentration, the diagnostic method by gold nanoparticles could be improved to establish itself as a standardized diagnostic method in medical as well as with environmental samples.

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# Activity of podophyllotoxin type lignans from *Bursera fagaroides var. fagaroides* against *Giardia lamblia* trophozoites.

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Abstract

Giardiasis, a diarrheal disease, is highly prevalent in developing countries, and has high prevalence rates particularly among young children. Several drugs are available for the treatment of this parasitosis, unfortunately, all of them presents variable efficacies and adverse effects, and some strains of *Giardia lamblia* have shown resistance towards common drugs. Studies on natural products have been a good strategy to discover new antigiardial compounds. *Bursera fagaroides*, species belonging to the family *Burseraceae*, is a flowering plant rich in lignans, terpenes and flavonoids. Ethanolic extracts of this plant have displayed potent cytotoxic activity against cancer cell lines and some parasites. However, the active molecule responsible for its antiproliferative/cytotoxic effect is not known.

Currently, fourteen podophyllotoxin-type lignans from Bursera fagaroides have been isolated and characterized, some of them have shown significant cytotoxic activity in several cancer cell lines. However, their effect on parasites has been poorly examined to date. In this study we analyzed the cytotoxic effect of 5′-desmethoxy-β-peltatin-α-methylether (5-DES), acetylpodophyllotoxin (APOD), burseranin (BUR) and podophyllotoxin (POD) on *Giardia lamblia* trophozoites. All tested lignans affected the *Giardia* adhesion. However, only 5-DES, APOD and POD exhibited growth inhibition. The effect of drugs was evident since the 12 h post-treatment, and the maximum effect was observed at 72 h (more than 70 % yield reductions of the parasites were detected). Concomitantly, microscopy images revealed morphological alterations after lignans treatment, 5-DES, APOD and POD provoked damage in the caudal region and ventral disk. With BUR any significant effect was observed in *Giardia* growth neither morphological alterations.

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# EFFECT OF ADIPOKINETIC HORMONS ON THE REGULATION OF ANTIMICROBIAL PEPTIDES IN *Anopheles albimanus* MOSQUITO.

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**Introduction:** The neuropeptides in insects are pleiotropic molecules and recently have been related to immune processes. They are involved in the expression of antimicrobial peptides and phagocytic activity in *Anopheles albimanus* mosquito. The adipokinetic hormones (AKH's) have been related with the extension of the phenoloxidase activity, enzyme involved in the pathogen melanization in hemolymph of insects. Our working group identified eighteen neuropeptides transcripts putative, in brain of *Anopheles albimanus* infected with *Plasmodium berghei*. Interestingly the AKH's I and II showed over-expression while the parasite was still in the midgut.

**Objective:** To know the effect of the neuropeptides in the expression of antimicrobial peptides in *An. albimanus* using an *in vitro* model.

**Methodology:** Midguts of mosquitoes were dissected and incubated in culture media added neuropeptides AKH I and AKH II. Media alone and lipopolysaccharide were used as controls. RNA was extracted, cDNA synthesized and the samples were analyzed by real-time PCR. All assays were made by triplicate.

**Results:** Data show an inhibition of gambicin and cecropin transcripts of 8 fold compared to control, in the presence of AKH's. When midguts were incubated with AKH I and LPS showed an expression of transcripts 1.39 fold compare to control.

**Conclusions**: Results suggest that adipokinetic hormones have an effect of down-regulation of antimicrobials genes in midguts tissue, but seem that the lipopolysaccharide revert this effect.

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# Expression of *Eh*CP1 in the bacterial periplasm and effect of the redox environment on its structure and function

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The intestinal protozoan parasite *Entamoeba histolytica* is the causative agent of human amebiasis, which is a highly prevalent infectious disease in developing countries. The life cycle of *E. histolytica* involves two main stages: trophozoite (invasive form) and cyst (infecting form). The major virulence factors of this pathogen are: (i) membrane proteins involved in cell adhesion, as the galactose-inhibitable lectin; (ii) pore-forming peptides that are inserted into the membrane of host cells, as the amoebapores; and (iii) secreted proteases implicated in tissue degradation, as the cysteine proteases (CP). Several proteases have been associated with invasiveness of *E. histolytica*, among them is the cysteine protease 1 (*Eh*CP1), which is a secreted protein that has the ability to degrade proteins of the extracellular matrix, allowing migration of the parasite to other organs (e.g., liver). The bacterial periplasm has two major proteins involved in the redox state of the compartment: DsbA, an oxidase that catalyzes the formation of disulfide bonds, and DsbC, a reductase-isomerase that is responsible for correcting mis-oxidized disulfide bonds. *Eh*CP1 has two non-consecutive disulfide bonds that are presumed essential for its structure and enzyme function. However, this notion has not been explored so far. Hence, we have taken advantage of the availability of a bacterial model to examine the effect of redox state on *Eh*CP1 functionality. Here, we report the outcome of expressing *Eh*CP1 in the periplasm of three *E. coli* strains: one wild-type and two null-mutants (dsbA and dsbC). To express and target *Eh*CP1 to the periplasmic compartment, its nucleotide sequence was inserted in-frame with the pelB signal peptide and cloned under the control of arabinose (BAD) promoter. The functionality of the enzyme was assessed by zymography and activity assays. Our results demonstrate that *Eh*CP1 is functionally active when expressed in the periplasmic compartment of the wild-type strain; however, reduced activity was detected in both mutant strains, indicating that the redox environment is affecting the structure (e.g., disulfide bonding pattern). Moreover, it seems that *Eh*CP1 depends on a foldase with isomerase activity to achieve its functionally active conformation. Studies of *Eh*CP1 oxidative folding assisted by an amebic PDI, a foldase with chaperone-like activity, are currently in progress.

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# Searching for a peptidic natural ligand of Aminopeptidase N/ CD13, a phagocytic receptor.

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## 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, the synaptic membranes of neurons in the Central Nervous System (CNS) and on myelomonocytic cells, such as monocytes, macrophages, dendritic cells and neutrophils. 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 for human coronavirus and cytomegalovirus, is involved in multiple processes, like angiogenesis, tumor cell invasion and migration, and has been shown to mediate signal transduction events leading to activation of MAPKs and cytoskeleton rearrangements. .

In human monocyte-derived macrophages it was shown that CD13 crosslinking increases the internalization of particles mediated by known phagocytic receptors (i.e. FcγR's). Also, it was recently demonstrated that CD13 can mediate phagocytosis by itself. Because of the many functions in which CD13 participates, it has been considered a moonlighting enzyme. However, much of the evidence for CD13 involvement in functions such as migration, phagocytosis, cell adhesion, has been obtained with the use of monoclonal antibodies (MoAb) and/or in genetically modified animals, since the natural ligands that could induce such 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 cell line HEK 293 was transfected with the *ANPEP* gene in order to express CD13 protein on its cytoplasmic membrane. This transfected cell line was used to select, from a commercial phage display library expressing 12 random amino acid sequences in the phage´s protein 3, phages with affinity to CD13. Different biopanning strategies were applied to isolate phages with amino acid sequences with affinity for CD13. Some of the selected phages were amplified separately and tested the interaction with CD13 and other irrelevant protein to be sure of the specific interaction. Finally, the positive clones were sequenced in order to determine the amino acid sequence responsible for the interaction with CD13. The sequences were compared between them in order to come up with a consensus sequence that could be present in the natural ligand of CD13.

## Results

The biopannings were made on HEK and HEK- ANPEP cells to enrich the population of positive clones to CD13. 93 clones were taken randomly and tested by ELISA.

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# Cloning, expression, and characterization of the recombinant mitosomal inorganic pyrophosphatase from *Entamoeba histolytica* (*Eh*IPP) and homology-based prediction of its three-dimensional structure

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The protozoan parasite *Entamoeba histolytica* is the etiologic agent of human amebiasis. Its most common clinical manifestations are amebic colitis and amebic liver abscess [1]. Interestingly, *E. histolytica* is a unique amitochondriate eukaryotic cell, having instead a membranous structure known as mitosome [2]. Recently, an inorganic pyrophosphatase (*Eh*IPP) was identified by proteomics analysis of these organelles [3]. To examine the function of *Eh*IPP, its coding sequence was amplified by PCR, using genomic DNA of *E. histolytica* HM1:IMSS as the template and two gene-specific oligonucleotides as primers. The PCR product was inserted into the bacterial expression vector pQE30 (Qiagen), yielding the recombinant plasmid pQE30*Eh*IPP. Two different strains of *E. coli* (ER2738 and SHuffle® Express) were used as a host for cloning and expression purposes. Initially, expression of recombinant *Eh*IPP in ER2738 rendered the formation of inclusion bodies. Further attempts at solubilization and refolding were unsuccessful. Conversely, expression of recombinant *Eh*IPP in SHuffle® Express yielded soluble protein under room-temperature conditions (RT), offering the possibility of protein purification under native conditions, performing the typical IMAC protocol (Qiagen). Biochemical characterization of the recombinant *Eh*IPP was performed using the standard IPPase assay. In addition, its tertiary structure was inferred by homology-based prediction and 3D modelling. Here, we report the results of the expression, purification, and characterization of the recombinant *Eh*IPP as well as the key features of the predicted three-dimensional model.

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# Gene silencing of an amebic protein disulfide isomerase (*Eh*PDI)

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In humans, amebiasis is a parasitic infection caused by the protozoan *Entamoeba histolytica*. Refereeing by reports from the World Health Organization, amebiasis is among the leading causes of mortality by infections with protozoans, producing about 70,000 deaths annually. The virulence of *E. histolytica* is characterized by its ability to secrete a vast assortment of molecules involved in tissue destruction and evasion of the immune system. Interestingly, the tertiary structure of some virulence factors is stabilized by disulfide bonding. In eukaryotic cells, protein folding and disulfide bond formation are performed within the endoplasmic reticulum, where a set of molecular chaperones and foldases assists the nascent polypeptide in acquiring their native conformation. Protein disulfide isomerase (PDI) is a foldase with a chaperone-like activity that plays a key role in the oxidative folding of polypeptides, by assisting the correct formation and rearrangement of disulfide bonds. The amebic PDI (*Eh*PDI, 38-kDa) has shown typical activities of a functional foldase, both *in vitro* (using standard assays) and *in vivo* (using heterologous expression systems). Due to the complex genetic features exhibited by this protozoan parasite, typical gene knockout methods are not feasible, forcing the use of alternative approaches for studying gene function. Here, we report the *Eh*PDI gene silencing by the RNA interference approach. The assessment of the molecular outcome is currently in progress, anticipating a significant decrease in gene expression that allows the establishment of the biological function of *Eh*PDI in the lifestyle *E. histolytica*. In addition, our approach will provide further evidence regarding the hypothesis that considers fundamental components of protein folding process as novel targets for the development of complementary drugs intended to treat and control the human amebiasis.

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# Profilin: Expression and identification of its ligands in *Trypanosoma cruzi*

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## Introduction

The eukaryotic cytoskeleton is a network of protein filaments involved in a wide range of cellular processes. The most conserved elements of the cytoskeleton are the microtubules and the microfilaments. The microfilaments are fibers of a globular protein named actin. The function and regulation of actin is regulated by a diverse set of proteins named actin-binding proteins (ABPs). In the protist parasite *Trypanosoma cruzi* actin is expressed, but its functional relevance and regulation has not been experimentally addressed. The analysis of the *T. cruzi* genome sequence revealed genes encoding for proteins homologous to ABPs. One of these genes encodes for profilin, a key regulator of actin polymerization dynamics in most cells. An important property of profilin is its capability to interact simultaneously with actin and several actin-regulatory proteins. For this reason, we chose this protein as a bait to identify novel actin regulatory proteins in *T. cruzi*.

## Results

To characterize profilin expression in *T. cruzi*, we raised poly-clonal sera against a GST fusion of profilin purified by affinity. Using these sera, we demonstrated the expression of the protein in insect and mammalian stages of the parasite by western blot assays. After this, we performed pull-down assays of parasite's protein extracts using recombinant GST-profilin as a bait and GST as a control. Samples from two independent pull-down experiments were analysed by LC-MS to identify parasite proteins bound to profilin. Six *T. cruzi* proteins were reproducibly identified in our assays with GST-profilin. They were absent in our GST controls, so they bind exclusively to the profilin portion of the recombinant protein. As expected, one of these proteins was actin. The remaining hits consisted in cytoskeletal proteins, translation factors, and mitochondrial and metabolic proteins. Additionally, 15 lower confidence profilin ligands were identified, including 5 hypothetical proteins.

## Conclusions

- Profilin is expressed in different life cycle stages of *T. cruzi.*

- We identified 6 high confidence profilin ligands that could represent actin regulatory proteins or reflect novel functions of actin in *T. cruzi*.

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# Characterization and detection of hydrolytic enzymes from *Toxoplasma gondii* RH strain in whole-cell extract and excretory/secretory products

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**Introduction:** *Toxoplasma gondii* is a cosmopolitan protozoan that has the ability to infect all nucleated cells from warm-blood organisms; this parasite affects about one-third of world population. After its infection, *T. gondii* is able to spread throughout the body and migrate across the biological barriers such as: intestinal, blood-brain and placental barrier, infecting immunologically privileged organs. To date, the mechanisms that the parasite uses to perform this process are unknown; however, in our laboratory we thought that the hydrolytic enzymes could play a crucial role as a virulence effector, and may be involved in parasite dissemination process. The aim of this study was to detect the proteases present in *T. gondii* isolated from cultured infected cell as well as from infected mice. **Methods:** We used a murine and *in vitro* model of toxoplasmosis and we detected the protease content in whole-cell extracts and in constitutive excretory/secretory (E/S) products from *T. gondii* tachyzoites of the RH strain using gelatin zymography and under different activation conditions such pH, cofactors and reducing agents. **Results:** Besides the standardization of the method for protease detection in *Toxoplasma*, we found, at least, nine proteases with capacity to degrade gelatin. They had a molecular weight range from 50 kDa to 290 kDa, all of them were sensitive to metal chelators, that indicate these proteases are metalloproteases. Additionally, we detected proteases in constitutive E/S products of the parasite, and we found al least five proteases with high sensitivity to chelators. Comparison of the content of proteases in whole-cell extract and E/S products from tachyzoites isolated from an *in vitro* model of toxoplasmosis and from the murine model; we found a diminished expression of proteases in the tachyzoites from infected cell cultures in contrast with the proteases of tachyzoites from infected mice. Interestingly the proteolytic activity was fully recovered when parasites maintained in cell culture were inoculated back into the *murine* model. **Conclusion:** The differential expression of these proteins could be related with the environment in which the parasite grows. In the murine model the parasite requires to transverse biological barriers and to fight against molecules from the immune response, a fact that does not happen in the cultured cell. Therefore, proteases might be function as virulence factor for the parasite.

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**Assessment of the involvement of protein kinase Eh-GSK3 in adhesion and phagocytosis of *Entamoeba histolytica*.**

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Amoebiasis is a parasitic infection of the intestines caused by the protozoan *Entamoeba histolytica*, pathogen that cause more than 50 million infections and 100 thousand deaths annually around the world, due to its high capacity of virulence that consists of a mechanism with three events: adhesion, cellular cytolysis and phagocytosis, which plays an important role for the survival of the pathogen. Recently, has been identified in the phagosomes of trophozoites to the kinase GSK3, and its known that the main component of the signaling system of the parasite consists of protein kinase, which are key regulators of the cellular function. Therefore, in this work we evaluated the expression of the protein GSK3 in trophozoites of *E. histolytica* and its participation in the adhesion and phagocytosis of the parasite**. Methods**: Molecular modelling to characterize the protein GSK3 *E. histolytica*. The expression of the gene *gsk3* was analyzed by RT-PCR. It was also realized assay of adhesion and phagocytosis of the trophozoites of *E. histolytica***. Results**: The molecular modelling showed that Eh-GSK3 presents 45% identity in the alignment of the amino acids and 90% of similarity in its tertiary structure with regard to GSK3β. The chemical inhibition of EH-GSK3 induced decrease in cell viability, activity of adhesion and phagocytosis and modified the organization of cytoskeleton of *E. histolytica*. **Conclusion**: The inhibition of EH-GSK3 affects the morphology and growth of the trophozoites, so that decreases the activity of adherence and phagocytic of *E. histolytica*.

Key words: *Entamoeba histolytica*, amoebiasis, phagocytosis, adhesion, GSK3.

# Two P2X1 receptor isoforms form heteromeric channels

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P2X receptors are membrane ion channels permeable to cations, which open in response to ATP binding. P2X subunits are encoded by seven genes (P2X1-P2X7) and form trimeric functional channels. ATP is an extracellular signaling molecule which it is recognized as a damage-associated molecular pattern and its release from dying or active cells modulates the innate immune system. P2X receptors are expressed in immune cells, thus, human monocytes express P2X1, P2X4 and P2X7 receptors.

Here we investigate if P2X1 receptors and its splicing variant (P2X1*del* ) are able to form heteromeric channels in single human monocytes.

Whole-cell recordings were made from monocytes isolated from peripheral blood, which were selected by their glass adherence properties. Amplification of the human P2X1 subunits was performed by nested PCR in single cells.

P2X1 mRNA was found in 90% of the monocytes analyzed and P2X1*del* was found in 88% of the monocytes. Currents mediated by P2X1 receptors in human monocytes showed an $EC_{50}$=6.3±0.2 µM and were inhibited (42%) by NF023, a P2X1 receptor inhibitor. At ATP concentrations of 10 µM and 30 µM, we recorded a rapidly desensitizing current, which was well fitted with a single exponential function (Tau=0.82± 0.23 s) and (Tau =1.45±0.19 s) respectively.

Our results indicate that the responses generated by ATP are due to P2X1 receptor or its variant splicing and that this two isoforms could form heteromeric channels.

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# PARTICIPATION OF THE HUMORAL AND CELLULAR ACTIVITY IN HEMOLYMPH OF CRAYFISH *Cherax quadricarinatus* CHALLENGE WITH β-1,3 GLUCAN.

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The main activity of the innate immune in invertebrates is to respond against pathogens through participation of cells that are located in the hemolymph (hemocytes).and humoral factors. Hemocytes are able to carry out phagocytosis, nodulation and/or encapsulation. Humoral factors such as the prophenoloxidase system (proPO), coagulation cascade and lectins participate in immune system. The use of components from pathogen microorganisms such as bacteria and fungi have been use to clarify the cellular and humoral response in invertebrates. In this report, we used β 1,3 glucan as bioestimulant. Hemolymph samples were taken at intervals of 0, 24, 48 and 72 h, we identified eight cell types based on Wright staining, differential count of hemocytes showed that roseate nucleocytes were the most abundant (45%). Total count of hemocytes indicated that in presence of β 1,3 glucan decreases the counting by 12,925 cells at 48 h post challenge, proteins concentration showed that the use of this biostimulant increased 8.92 mg/ml compare to control at 24 h. *C. quadricarinatus* lectin (CqL) exhibited the highest specific activity in presence of rat erythrocytes at 24 h in the three groups (control, saline solution, and β-1,3 glucan). ProPO enzyme was observed in all cell populations and when we challenged with β-1,3 glucan, melanization in the cells were reduced compared to control group. The quantification of CqL by ELISA in the β-1,3 glucan group was 0.036% and in the group control was 0.058% of the total proteins. Our results suggest that immune responses depend of the specific recognition of PAMPs. Humoral and cellular factors were involved in the innate immune response at different times, the reaction to β-1,3 glucan increases the concentration of the serum lectins and the total number of hemocytes; in relation to proPO in presence of the biostimulant, we found that exist a direct relationship between the respond of hemocytes and hemolymph. PAPIIT IN 214315 UNAM, MEXICO.

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# Hsa from *Streptococcus gordonii* induces the priming of neutrophils

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## Abstract

Neutrophils are the most abundant circulating leukocyte in humans, represent 50-70% of circulating leukocytes, and the first to respond against extracellular pathogens. Neutrophils migrate from circulation to foci of infection in response to bacterial or host-derived chemoattractants. Neutrophils ensnare and destroy invading pathogens by degranulation, phagocytosis, production of reactive oxygen species (ROS), and/or deploy of neutrophil extracellular traps (NETs). These activities limit the spread of pathogens to sterile sites including the bloodstream, lung, etc. *Streptococcus gordonii* is commensal Gram-positive bacterium that colonizes the human oral cavity. After tooth extraction surgery, *S. gordonii* can cause systemic infection resulting in infective endocarditis. Hsa is an adhesin that plays a significant role in dental plaque development and recognition of cell surface receptors on leukocytes. In monocytes, Hsa induced the secretion of pro-inflammatoy cytokines and maturation into dendritic cells. Since neutrophils are relevant immune cells against extracellular bacterial infection, we evaluate if Hsa may activate neutrophils by determining the expression of the activation marker CD11b, L-selecting shedding (CD62L), FcγRI expression (CD64), reactive oxygen species production and formation of DNA-based neutrophil extracellular traps (NETs). Our findings are relevant to immune response against extracellular bacteria and pathological processes of infective endocarditis.

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# P2X receptors in human macrophages

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P2X receptors are found in a wide variety of tissues where they appear to be involved in a variety of physiological processes. Experimental data indicate that human macrophages express P2X1, P2X4 and P2X7 receptors, however, there are not electrophysiological studies that characterize their properties in these cells. Therefore, the aim here was to study the currents mediated by P2X receptors in human macrophages, using the whole cell configuration of the Patch Clamp technique. Human monocytes of healthy voluntaries were cultivated for 15 days to generate macrophages, which were stimulated with ATP and their membrane currents recorded. The amplitude of these currents were increased dependently of ATP concentrations (0.3 to 100 µM). The ATP concentration to induced half of the maximal responses ($EC_{50}$) was 3.1 µM in the general macrophage population. Currents recorded in response to concentrations lower than 100 µM decreased rapidly despite the continuous presence of the agonist. This desensitization was well fitted to an exponential function. With higher ATP concentrations (3-5 mM), a second phase was noticed when the amplitude of these currents increased during the continuous presence of ATP. These results, together with recent observations in monocites, suggest that P2X1 receptors are involved in mediating these currents in macrophages.

**KEYWORDS:** Macrophage, P2X receptors, Macrophage phenotype M1, Macrophage phenotype M2, ATP.

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# Effect of the VSP9B10A protein from *Giardia duodenalis* on the intestinal epithelium, using gerbils (*Meriones unguiculatus)* as experimental model of giardiasis.

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*G. duodenalis* is the causative agent of giardiasis, a worldwide distributed parasitic infection. An initial step in the pathophysiology of this disease is the trophozoite adhesion to the epithelial cells of the small intestine. This process involves different parasite structures and proteins, such as variable surface proteins (VSPs). Our group has identified a novel function of the VSP9B10A, it has a proteolytic activity and causes damage in epithelial cell monolayers. The aim of this study was to determine the effect of this protein in an experimental model of giardiasis in gerbils (*Meriones unguiculatus*) In this animals were inoculated with transfected trophozoites that stably express the VSP9B10A$^{+}$ and as a control we used the trophozoites that do not express it (VSP9B10A$^{-}$) or PBS. Initially, the distribution of VSP9B10A$^{+}$ and VSP9B10A$^{-}$ trophozoites in the small intestine was determined and the damage to intestinal sections using histological sections that were stained with H & E and PAS. Also, indirect immunofluorescence assays were performed to detect the production of MUC-2. The results showed that in both cases the trophozoites localized in the duodenum. The effect of the VSP9B10A$^{+}$ trophozoites on the intestinal epithelium was characterized by damage in brush border, enterocytes flattening, pseudostratification and goblet cell hyperplasia. These changes were more marked in gerbils inoculated with VSP9B10A$^{+}$ trophozoites as compared with the ones observed in gerbils inoculated with VSP9B10A$^{-}$ trophozoites. MUC-2 production was higher in experimental animals and showed a differential distribution, compared to control animals. All together these results suggest that trophozoites expressing the VSP9B10A$^{+}$ protein induces damage to the intestinal epithelium thus this may be considered as a virulence factor. Currently we are analysing the induction of immune response in the intestinal epithelium in control and experimental animals using RT-PCR to determine the expression of TNF-α, IFN-γ, IL-6, IL-1β, IL-10, KC and GAPDH genes.
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# Gene expression and cytotoxicity of *Trichomonas vaginalis* during interaction with prostatic cells mediated by $Zn^{2+}$

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*Trichomonas vaginalis* infects the urogenital tract of humans causing trichomoniasis, a sexually transmitted infection that increases the susceptibility to HIV infection and the development of cervical or prostate cancer. It has been recently evaluated the effect of vaginal microenvironment components as $Fe^{2+}$ in the adhesion and cytotoxicity of this parasite to epithelial (VECs) and cervical (HeLa) cell lines. Few studies address male trichomoniasis; therefore, this work evaluates the effect of $Zn^{2+}$ (1.6 mM) on the cytotoxicity and gene expression of *T. vaginalis* during their interaction with the prostatic cell line DU145 in the presence of $Zn^{2+}$. The results showed that $Zn^{2+}$ significantly affects the expression of *ap65*, *ap33*, *gapdh*, *enolase*, *tvcp39*, *mp50* and *tvfim1* of *T. vaginalis* and reduce their cytotoxicity to prostatic cells. These data provides evidence that the male urogenital microenvironment has an effect on the expression of virulence factors of *T. vaginalis*.

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# Expression of actin 2, an actin variant of *Trypanosoma cruzi*, and identification of some of its ligands.

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BACKGROUND: Trypanosomatid parasites are characterized by an atypical cytoskeleton mainly composed by microtubules. The presence of a conventional actin similar to that found in animals and plants have been demonstrated in these parasites, but no microfilaments have been detected by electron microscopy. Interestingly, the bioinformatic analysis of the *T. cruzi* genome identified three other genes encoding for putative actins. Of these, Actin 2 (A2) has a 51% identity with conventional actin (A1) and it is not found in *T. brucei* or *Leishmania*. Here, we studied the expression of A2 in different stages of *T. cruzi*, and identified some of its ligands by pull-down assays and mass spectrometry.
METHODS: Expression mRNA of A2 was analyzed by PCR using a cDNA library as template. Specific polyclonal antibodies were produced using a recombinant GST-A2 fusion protein and used in western blot and immunofluorescence assays. The recombinant proteins were also used to perform pull-down assays with soluble extracts from epimastigotes. The identification of these proteins was determined by mass spectrometry (MS).
RESULTS: A2 gene is expressed at the mRNA and protein levels. The protein is detected in epimastigotes, amastigotes and trypomastigotes with no apparent differences in expression levels. In epimastigotes, immunofluorescence assays showed a speckle pattern all over the parasite, unlike the pattern observed for A1, which is expressed as patches in the flagellum and as a dense concentration near its base. Preliminary results from pull-down assays using the recombinant fusion proteins GST-A1 and GST-A2 did not show differences in the profile of binding proteins between the two actins. Interestingly, none of the interacting proteins identified by MS correspond to canonical actin binding proteins. However, some of the proteins detected, such as EF1a and GAPDH, have been demonstrated to interact with actin in other organisms.
CONCLUSIONS: *T. cruzi* actin 2 is a functional gene that is expressed as protein throughout the life cycle of the parasite. Differences in subcellular localization suggest functional differences between A1 and A2. We have identified some novel actin ligands in the parasite. However, we found no differences in the proteins that bind A1 and A2. We are expressing EGFP fusions in vivo to identify proteins that bind specifically to these actins.

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# Effect of a Recombinant Probiotic on Lung Inflammation in an Experimental Asthma Model

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INTRODUCTION: Allergic asthma is an airway inflammatory disease induced by Th2 immune response and characterized by eosinophilia. Probiotics and prebiotics have shown important roles as immunomodulators in allergies.

OBJETIVE: To evaluate the effects of oral administration of a recombinant probiotic that produces murine IL-22 (L22), alone or combined with a dairy bioactive peptide (DBP) on the inflammatory response in asthmatic rats.

MATERIALS AND METHODS: For asthma model, five groups were established as follows: a Sham groups and four asthmatic groups which were treated or untreated with L22, L22+DBP or DBP along the induction asthma protocol. Cellularity in bronchoalveolar lavage (BALF) and eosinophilia in lung tissue were analyzed. Collagen type I deposition was measured by Sirius red staining in the lung. Also, the relative expression of Th1, Th2 and Treg cytokines by qPCR were determined in lung tissue.

RESULTS: Total cellularity was diminished (44.8%) when L22 was administered. Also, number of eosinophils and neutrophils were reduced (79.3% and 53.7%, respectively) in BALF. Otherwise, eosinophilia in lung tissue was reduced in 39.8%. A significant reduction of collagen was observed in the lung tissue. Treatment with L22 significantly diminished the expression level of IL-5 (57.6%) e IL-13 (77.2%), and promoted the increment of IFN-gamma, IL-10 and TGF-beta (6.1, 8.9 and 8.9-fold, respectively). No improvement of immune attenuation was achieved when L22 was co-administered with DBP, but increased expression of IL-10 in lung tissue was observed as compared with only DBP administration.

CONCLUSIONS: The treatment with L22, alone or in combination with DBP, induces a reduction of asthma-associated Th2 response in the lung, likely through of an unbalance to Th1 and Treg responses.

KEY WORDS: allergic asthma, probiotic, immunomodulation.

# B cells respond via germinal centers to produce anti-lipid IgG antibodies

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Anti-lipid IgG antibodies are produced in some mycobacterial infections and autoimmune diseases (lupus, anti-phospholipid syndrome). However, few studies have addressed the mechanisms that lead to the production of these immunoglobulins. Anti-lipid IgG antibodies are consistently found in a mouse model of lupus induced by chlorpromazine-stabilized non-bilayer phospholipid arrangements (NPA). NPA are transitory lipid associations found in the membrane of most cells; when NPA are stabilized they can become immunogenic and induce specific IgG antibodies, which are involved in the development of the disease. We used this model of lupus to investigate the *in vivo* mechanisms that lead to the production of anti-lipid IgG antibodies. B cells are activated, in response to most protein antigens, via germinal centers or extrafollicular reactions. In germinal centers, a T cell-dependent response leads to isotype switch, somatic hypermutation, affinity maturation and memory generation, whereas these events do not usually occur in extrafollicular reactions. In this mouse model of lupus induced by chlorpromazine-stabilized NPA, we found plasma cells producing NPA-specific IgGs in the inguinal lymph nodes, the spleen, and bone marrow of mice. We also found a significant number of germinal center B cells specific for NPA in the inguinal lymph nodes and the spleen, and we demonstrated the presence of NPA in these same germinal centers. In contrast, we found very few extrafollicular reaction B cells specific for NPA. Altogether, our data suggest that, in this murine model of lupus, B cells produce anti-NPA IgG antibodies mainly via germinal centers.

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# The Role of Autophagy During the Mouse Neural Tube Closure

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Autophagy is a lysosomal catabolic process that sometimes promotes type II programmed cell death, and mediates protein traffic to plasma membrane and non-conventional secretion. Autophagy has been associated with many processes throughout mammalian development, from implantation to osteogenesis, but their role during early neural development is still unclear.

In vertebrate development the primordium of central nervous system forms in a process known as neurulation. In this process, at the dorsal part of the embryo, the neuroectoderm in proximity to the notochord thickens and flexes at a medial hinge point, forming two concave walls that extend along the embryo's anterior-posterior axis. Later, the dorso-lateral portion of the walls bent causing their tips to meet at the midline and fuse forming a hollow cylinder known as neural tube. When the neural tube does not form properly neural tube defects occur. Among these disorders, anencephaly and spina bifida have the highest incidence in newborns. Although the absence of activating autophagy protein (AMBRA1) causes lethality and neural tube defects in mouse embryos, no study has proven whether autophagy participates in the closing of the neural tube.

In this work we demonstrate the presence of abundant cells with high autophagic activity along the fusion line of the neural tube of mouse embryos. Furthermore, ex-utero culture of early embryos in the presence of the autophagy inhibitor Spautin-1 caused neural tube closure and embryo turning defects. These data suggest that autophagy contributes to neural tube closure in the developing mouse.

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# Activation of hippocampal adult neurogenesis in response to a context-fear memory task after an excitotoxic focal dentate gyrus lesion

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Adult hippocampal neurogenesis occurs in the adult dentate gyrus (DG) of the hippocampus. Neurogenesis modulates contextual fear memory and memory extinction. Beyond its role in memory, adult neurogenesis has gained attention as a possible mechanism for neuronal repair since it increases after brain damage. However it remains unclear if new neurons are involved in neurorepair processes. We have previously shown that a morphological reorganization as well as the functional (behavioral and electrophysiological) restoration of lost hippocampal DG function occurs along time after a focal DG lesion. Now we ask if neurons born after damage: 1) mature along time and; 2) show activation in response to a hippocampal dependent task. We induced a focal excitotoxic lesion in the DG and analyzed at two time-points: 1) learning and memory in a contextual fear task; 2) cell proliferation and maturation and; 3) c-fos expression in new-born neurons after a contextual fear memory task. In this work we observed that at 10dpl memory retrieval is impaired, but not at 30dpl, which correlates with the previous observed anatomical reorganization. At 10dpl there is an increase in the number of young new neurons (BrdU+/DCX+) in both the GCL and hilus, moreover, the number of mature new neurons (BrdU+/NeuN+) is bigger than sham group, suggesting a possible acceleration in the maturation process after lesion. We observed an increase in the number of young and mature new neurons prone to activate (BrdU+/DCX+/c-fos+ and BrdU+/NeuN+/c-fos+) in the GCL, nonetheless the functional recovery is only observed at 30 but not at 10dpl, suggesting that new neurons are not necessarily involve in functional recovery after damage. The activation of new neurons (showed by c-fos expression) at an immature state (10dpl) could suggest an acceleration in the maturation process or a modification in the activation threshold of these neurons after damage in response to a contextual fear memory task. However we can not rule out an aberrant activation. At 30dpl the most of the new cells (BrdU+) are mature neurons (BrdU+/NeuN+) and the number is bigger after damage than in sham group, this means that neurogenesis remains increase a month after lesion, this effect is only observed in the GCL but not in the hilus.

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# Sympathetic fat denervation improvement the metabolic alteration caused for Sleep restriction

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Circadian disruption is associated with metabolic disturbances such as hepatic-steatosi, obesity and type-2 diabetes. The rhythm of current society has led to less quality and time of sleep and rest, causing a over activation of Hypothalamus Pituitary Adrenal (HPA)and Locus Coeruleus Norepinephrine (LC-NE) axis. This could result a increases of Sympathetic Nervous System activation and the liberation of cortisol. Promoting metabolic syndrome, specifically modified lipid metabolism and insulin resistance. We hypothesized that Sleep Restriction (SR) induced metabolic alteration and obesity in part mediated by the enzyme 11β-HSD1 synthesized in the adipocyte and the sympathetic denervation (SRDx) of adipose tissue improves the alterations in animals with chronic stress by sleep restriction. We use wistar rats divided in four groups (SR, CTL, SRDx and CTLDx). Two groups (SDRx and CTLDx) underwent surgical denervation and placement of device measuring body temperature prior to beginning sleep restriction protocol (18hrs waking and 6hrs rest from Monday to Friday) for 8 weeks. Weighing animals, food intake and blood samples were taken every week. Every two weeks in metabolic cages were placed and in the 4 and 8 weeks was performed intraperitoneal glucose tolerance test (IPGT). At end of 8 weeks the animals where sacrificed and epididymus, retroperitoneal adipose tissue, liver and pancreas was collected and kept at -80C for the PCR analysis and then the animal was prefunded and the tissues were placed in paraformaldehyde 4%.

We found that SR don't cause changes in body weight and food intake in all groups after 8 weeks of protocol. However the weight gain in Dx groups is minor although they feed more. The temperature analysis shows a decrease in animals with SR during the active phase, this phenomenon doesn't be observed in SRDx. In the SR animals the IPGTT showed an increase in the glucose level in the 8 weeks, suggesting glucose intolerant, these changes in SRDx animals don't were observed. We can conclude than fat denervation prevents dysregulations in glucose caused by Sleep restriction, besides had an effect in body temperature caused a decrease in this parameter during normal activity phase.

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# Nicotine effect in a Parkinson's disease model induced by human α-Synuclein and Synphilin expression in *Drosophila melanogaster*

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Parkinson's disease (PD) is the second most common neurodegenerative disease only after Alzheimer's disease. There are genetic and environmental factors in the development of this disease being the aging the principal cause. One of the main characteristics in PD is the death of dopaminergic neurons in a brain region called *substantia nigra pars compacta*. PD continues to increase as one of the main health problems in terms of disability and mortality in worldwide.

There is evidence that nicotine has neuroprotective properties, and that the risk of PD in smokers is slightly lower than in the general population. Nicotine is an antioxidant that could slow the effects of neuronal degradation and the loss of function of the dopaminergic neurons.

We evaluated the effect of nicotine in a PD model induced by two human proteins, α-Synuclein and Synphilin, which are expressed in *Drosophila melanogaster* dopaminergic neurons under control of UAS-GAL4 system. In a PD Drosophila model the expression of α-Synuclein or Synphilin induced a decrease lifespan associated with the progressive loss of dopaminergic neurons. Therefore survival curves were performed to determine if different nicotine concentrations suppress neurotoxicity. Additionally we evaluated the flies motor deficits performed climbing assays.

Interestingly on the one hand we have shown decrease lifespan and increase in a motor deficits in control flies but on the other hand we have shown increase in lifespan and decrease in motor deficits in the 2.4 µM nicotine concentration in α-Synuclein and Synphilin flies. We concluded that nicotine only causes a beneficious effect in parkinsonian flies being toxic for control flies.

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# *In vitro* and *in vivo* neuroprotective activity of *scammonin 1* and *tyrianthin C* isolated from root of *Ipomoea tyrianthina*

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**Introduction.** Mexico possesses a great diversity of plants that have been employed in traditional medicine. Several plants of the family Convolvulaceae have been used for the treatment of neurological disorders. Organic extracts and pure metabolites obtained from the root of *Ipomoea tyrianthina* have been reported with pharmacological properties on the central nervous system (CNS), such as the release of γ-aminobutyric acid (GABA), in addition to sedative and anticonvulsant actions. These results suggest that some glycolipids might produce neuroprotection at histological level. Since the GABAergic system represents one of the intrinsic mechanisms of neuroprotection, it has been considered as a target of some metabolites with anticonvulsive activity. The aim of this project was to evaluate the effects of the glycolipids scammonin 1 and tyrianthin C on the CNS of mice, linking them with *in vitro* GABA release and *in vivo* anticonvulsant and neuroprotective activities in an acute pentylenetetrazole (PTZ) seizure model. [1-5] **Methods.** Scammonin 1 and tyrianthin C were isolated from the root of *Ipomoea tyrianthina.* Structure elucidation was performed by 1D and 2D Nuclear Magnetic Resonance (NMR) and mass spectrometry.[4,5] Scammonin 1 and tyrianthin C *in vitro* assays were made using cerebral cortex slices from CD1 mice (20-30 g).[6] For *in vivo* evaluations, mice were administered intraperitoneally with scammonin 1 or tyrianthin C at doses of 40, 80, and 120 mg/kg, in an acute scheme by means of a PTZ (70 mg/kg) seizure model to estimate the anticonvulsant and neuroprotective actions on cerebral cortex. [7] **Results.** For *in vitro* experiments, both compounds evoked endogenous GABA release and increased its concentration within the incubation medium compared with controls; *tyrianthin C* demonstrated a dose-dependent effect. Sodium absence and guvacine presence did not affect significantly the activity of glycolipids tested, in contrast with the calcium-free and 2-hydroxisaclophen presence mediums, where GABA concentrations were considerably reduced. When evaluated *in vivo*, the acute administration of *scammonin 1* and *tyrianthin C* decreased the number of seizures and increased the latency with respect to the administered doses, showing 16.7%, 66.7% and 100% of convulsion protection rates. Both compounds lessened the neuronal alterations and the interstitial edema generated by PTZ induction at the doses assessed. **Conclusions.** *Scammonin 1* y *tyrianthin C* showed anticonvulsant and neuroprotective effects in the PTZ model. In addition, both compounds increased the release of GABA, possibly through modulation of vesicular GABA release or GABA elimination processes. **Acknowledgements.** We thank the CONACyT for financial support (Grants 90268 and CB2011-168569) and a graduate student scholarship (43923). **References.**
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# MODULATION OF GENE EXPRESSION OF THE REST/NRSF COMPLEX BY TIME-RESTRICTED FEEDING IN A PHARMACOLOGICAL SEIZURE MODEL

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The repressor element-1 silencing transcription factor (REST) or neuron-restrictive silencer factor (NRSF), is known to play a master role in neuronal cells and their dysfunction has been implicated in neurological and neurodegenerative diseases. REST/NRSF operates as a scaffold protein because of the C-terminal domain recruits CoREST and histone deacetylases 1 and 2 (HDAC 1/2). This complex is essential to repress the transcription of large numbers of genes containing RE1 sites. Recently, it has been described an increase of both gene and protein expression of REST/NRSF after *status epilepticus* (SE) induction, whereby it is hypothesized that this factor could be involved during epileptogenesis process. On the other hand, our laboratory has described that a restrictive diet such as time-restricted feeding (TRF), had an anticonvulsant effect in the pilocarpine-seizure model and such effect was mediated by changes in metabolic signaling pathways (AMP-activated protein kinase) and epigenetics marks (increased histone 3 acetylation). The aim of the present work was investigated whether the TRF could be modulating (downregulate) the gene expression of REST/NRSF-associated components such as REST, CoREST and HDACs 1/2. Briefly, one group of rats had free access to food and water *ad libitum* (AL) and a second group underwent a TRF schedule that consisted of allowing rats to feed freely for only 2 h daily for 20 days, then, we proceeded to induce SE with pilocarpine injection. Our preliminary data showed an increase of REST/NRSF gene expression after 24 h of SE-induction in *ad libitum* fed animals (n=5) (AL-SE) compared with control groups (AL). Also, we observed a decrease in REST/NRSF expression in TRF fed animals after SE induction (TRF-SE) compared with AL-SE rats, however, it was not statistically significant. Interestingly, similar results were observed in CoREST, and HDACs 1/2 genes because there was an increase in their expression in AL-SE fed animals and a decrease in rats that were subjected to TRF schedule. These results confirmed that REST gene expression is increased after SE induction and even more, showed that other components of REST/NRSF complex are increased after seizures. Most important, in TRF animals a decrease in their gene expression was observed suggesting that restrictive diet is able to downregulate components of REST/NRSF complex and thus, it would explain in part, a new mechanism involved in the anticonvulsant effect of TRF previously reported. This project was supported by CONACYT 239595 grant.

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# The recombinant five disulfide-bonded spider toxin rOxyTx1 is an insecticide peptide that block calcium channels in cockroach dorsal unpaired median (DUM) neurons

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The gene of the five disulfide-bonded peptide toxins 1 (named Oxytoxin or Oxotoxin) from the spider *Oxyopes lineatus* were cloned into the expression vector pQE30 containing a 6His-tag and a Factor Xa proteolytic cleavage region containing the residues IERG. A fusion protein was expressed in *Escherichia coli* BL21 cells under induction with isopropyl thiogalactoside (IPTG). The recombinant product was named HisrOxyTx1 and the protein expression was *ca* 14 mg/L of culture medium. The recombinant toxin HisrOxyTx1 was found exclusively in inclusion bodies, which were solubilized using a chaotropic agent, and then, purified using affinity chromatography and reverse-phase HPLC (RP-HPLC). The HisrOxyTx1 product, obtained from the affinity chromatographic step, showed several peptide fractions having the same molecular mass of 9,913.1, indicating that HisrOxyTx1 was oxidized forming several distinct disulfide bridge arrangements. The isoforms of HisrOxyTx1 after DTT reduction eluted from the column as a single protein component of 9,923. *In vitro* folding of HisrOxyTx1 yielded single oxidized component, which was cleaved by the proteolytic enzyme Factor Xa to give the recombinant peptide rOxyTx1. The experimental molecular mass of rOxyTx1 was 8,059.0 and 6,176.4 Da, which agree with its expected theoretical mass. The recombinant peptide rOxyTx1 showed lower but comparable toxicity to the native toxin when injected into lepidopteran larvae; furthermore, rOxyTx1 was able to inhibit calcium ion currents on dorsal unpaired median (DUM) neurons from *Periplaneta americana* cockroaches.

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# *Malva parviflora* immunomodulatory effect in a mouse model of Alzheimer´s disease.

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Alzheimer´s disease (AD) is a neurodegenerative disorder characterized by neurofibrillary tangles (NFT) and senile plaques (SP), two hallmarks that lead to a progressive loss of memory and cognitive disfunction. Recent studies have established a chronic inflammatory process and an oxidant state with the progression of AD. Epidemiological studies have shown a decreased incidence of AD in people with prolonged treatment of NSAIDs. Currently, the use of standardized extracts obtained from plants have been proposed as potential therapeutic tools to treat AD based on their chemically diverse constituents including those with anti-inflammatory properties. So that, here we tested 4 different derivatives of *Malva parviflora* (dichloromethane extract – MpD-, Fraction 7 –MpF7- , Fraction 10 – MpF10- and Fraction 13 – MpF13-), a Mexican plant with anti-inflammatory and neuroprotective properties (Aslam M. *et al*, 2014; Jimenez-Ferrer. *et al* 2016). We demonstrated that, contrary to MpF7, MpD, MpF10 and MpF13 possess immunomodulatory properties as they significantly decreased the activation of NF-kB and AP-1 in a dose-dependent manner in macrophages stimulated with LPS. Besides this, MpF10 showed a neuroprotective effect in an LPS-induced AD murine model.
Current experiments are performed to determine the chemical composition of these derivatives.
This work was partially supported by grants from DAGPA/UNAM (IN213316 and IN212316), from CONACYT (155290 and 154542) and from IMSS (FIS/IMSS/PROT/G11-2/1018).

Keywords: inflammation, Alzheimer's disease, *Malva parviflora*,

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# Isoforms of REST in vertebrados evolutionarily distant vertebrates

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**ABSTRACT**

REST is a zinc finger protein considered a neurogenesis master regulator in mammals. The tissue-specific levels of REST alterations usually trigger cancerous processes. So far it is unknown whether the evolutionary origin of REST took place in organisms with rudimentary nervous systems, and does not have a prototype of the superfamily of zinc finger proteins that could be the molecular ancestor of REST. Our *in silico* analyses show high conservation of REST *DNA Binding Domain* (DBD) and low conservation in *Represor Domain 1* (RD1), whereas te Represor Domain 2 (RD2) appears later in mammals. In our interpretation, the origin of a rudimentary REST capable to contact DNA, dates back to a time prior to the emergence of modern fish (500 m. A.), while the represor activity of modern isoforms were acquired during the onset of reptiles and specialyzed in mammals. We identified experimentally various isoforms of REST in extracts of skeletal muscle tissue of vertebrate organisms. Western blot and Immunoprecipitation results showed an isoform of 150-250 kD in *Iguana iguana*, *Streptopelia decaocto* and *Peromyscus mexicanus*, which matches the weight of the canonical isoform. Interestingly two bands of 71 and 37 kDa are present in all organisms studied. In addition, in *P. mexicanus* a variety of short isoforms were found, suggesting that new isoforms were acquired in vertebrates approximately 200 m.y.a. This work is the first experimental evidence in finding REST isoforms in evolutionarily distant animals and represents an initial step in the reconstruction of the natural history of this protein.

**Keywords:** REST isoforms, Evolution, Nervous System.

# Effect of Krill oil in the hippocampus of adult rats with seizures induced by pentylenetetrazole and febrile seizures at five postnatal days.

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Epilepsy is an alteration of the brain that is characterized by the sustained predisposition to generate epileptic seizures, accompanied by social consequences, psychological, cognitive and neurobiological. Epilepsy affects some 67 million people around the world and 80% of the patients live in developing countries. For that reason, it is important investigate new treatment options.

Krill oil (KO) is one of the best sources of polyunsaturated fatty acids (PUFAS), in particular omega-3 and antioxidants, showing a neuroprotective role. Promising results have been achieved against neurodegenerative processes and loss of cognition with its use. In this work we evaluated the effect of krill oil in the hippocampus of rats with seizures induced by pentylenetetrazole in order to determine its effectiveness as neuroprotective oil.

For this purpose, male Wistar rats with chronic intragastric administration of Palm oil (PO, 300 mg/kg, n=4, control group), water (1 ml, n=4, control group) and KO (300 mg/kg, n=6, experimental group), starting at birth and until 40-60 postnatal days (PD) were used; these animals had febrile seizures induced by hyperthermia at 4-5 PD. Also, animals of each group were induced seizures with pentylenetetrazole (PTZ, 90 mg/kg i.p) approximately 40-60 PD.and were followed by intracardiac perfusion with paraformaldehyde (4%) and removed the brains. Brain slices of 30µm were processed by Immunofluoresce against GFAP (glial fibrillary acidic protein, astrocytes) and NeuN (neuronal nuclei, neurons). This was followed by a cellular quantification of the regions of hippocampus (CA1 and CA3) and dentate gyrus (DG).

The treatment with KO and PO increased the number of astrocytes compared with rats treated with water in CA3 (20.61±1.189 and 19.83±2.040 vs. 13.62±1.286, p=0.006, respectively) and DG (22.08±1.864 and 19.67±1.229 vs. 15±1.151, p=0.016, respectively). Also, the results showed an increase is the number of neurons in the group treated with KO and PO in comparison with the water group in DG (110.3±11.16 and 111.6±8.042 vs. 68.95±7.896, p=0.0119, respectively).

In conclusion the chronic treatment of KO and PO showed some neuroprotection especially in DG during seizures. However, the KO did not show greater efficiency than the PO even if a different concentration of PUFAS and antioxidants has.

El trabajo corresponde a la especialidad de Neurociencias, Epilepsia.

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# Characterization of TRPV1 glycosylation

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TRPV1 receptor (Transient Receptor Potential Vanilloid 1) is a cationic ligand-gated ion channel with higher permeability for Ca2+. It is predominantly expressed in dorsal root ganglia as well as in the trigeminal ganglion neurons where has a role as a molecular detector of noxious stimuli such as high temperature (>42 °C), irritant compounds, pH changes, lipids, amines and other molecules release during tissue damage and inflammatory processes. This ion channel is also regulated by post-translational modifications; besides phosphorylation and covalent modifications of cysteine residues, TRPV1 undergoes glycosylation (N604) which is a crucial modification for protein biosynthesis and folding. Particularly, N-glycans have been found to regulate the trafficking of ion channels in the nervous system, moreover extracellular sialic acid residues, which are negatively charged at physiological pH, influence the sensitivity of the voltage sensor domains to the transmembrane electrical potential difference.

In this work, we demonstrate that TRPV1 glycosylation is an species-dependent post-translational modification. We also deeply characterized the N-glycosylation state of the TRPV1 ion channel and demonstrate that consensus tripeptide (Asn-Xaa-Ser/Thr) on TRPV1 sequence is important for degree branching of its N-glycosylation.

There is still so much to identify about the structural properties of the channel and to a large extent of work for knowing the impact of post-translational modifications, such as the N-glycosylation on the TRPV1 ion channel physiology.

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# Glycyrrhizin ameliorates oxidative stress and inflammation in hippocampus and olfactory bulb in lithium/pilocarpine-induced status epilepticus in rats.

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Glycyrrhizin (GL), is a triterpene present in the roots and rhizomes of *Glycyrrhiza glabra* that has anti-inflammatory, hepatoprotective and neuroprotective effects. Recently, it was demonstrated that GL produced neuroprotective effects on the postischemic brain as well as on the kainic acid injury model in rats. In addition to this, GL also prevented excitotoxic effects on primary cultures. The aims of the present study were to evaluate GL scavenging properties and to investigate GL's effect on oxidative stress and inflammation in the lithium/pilocarpine-induced seizure model in two cerebral regions, hippocampus and olfactory bulb, at acute time intervals (3 or 24 h) after *status epilepticus* (*SE*).

Fluorometric methods showed that GL scavenged three reactive oxygen species: hydrogen peroxide, peroxyl radicals and superoxide anions. In contrast, GL was unable to scavenge peroxynitrite, hydroxyl radicals, singlet oxygen and 2,2-diphenil-1-picrylhydrazyl (DPPH) radicals suggesting that GL is a weak scavenger.

Additionally, administration of GL (50 mg/kg, i.p.) 30 min before pilocarpine administration significantly suppressed oxidative stress. Moreover, malondialdehyde levels were diminished and glutathione levels were maintained at control values in both cerebral regions at 3 and 24 after SE. At 24 h after SE, glutathione S-transferase and superoxide dismutase activity increased in the hippocampus, while both glutathione reductase and glutathione peroxidase activity were unchanged in the olfactory bulb at that time.

In addition, GL suppressed the induction of the proinflammatory cytokines interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) in both cerebral regions evaluated. These results suggest that GL confers protection against pilocarpine damage via antioxidant and anti-inflammatory effects.

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# Role of autophagy in the physiological aging of the brain and the establishment of a senescent phenotype

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Autophagy is mainly a catabolic process that engulfs aberrant organelles, misfolded proteins and protein aggregates into double membrane vesicles (named autophagosomes), which then fuse with lysosomes. The correct function of this catabolic process is very important because it is the only known mechanism that eukaryotic cells posses to degrade protein aggregates and entire organelles, such as mitochondria and peroxisomes. Post-mitotic cells like neurons are highly dependent on autophagy because they lose the ability to dilute insults by cell division. Neuronal health heavily depends on housekeeping processes to maintain cellular quality control, as loss of autophagy causes accumulation of ubiquitin-positive inclusion bodies and triggers a process of neurodegeneration. During aging, decreased autophagic activity has been extensively reported. In an *in vitro* model of primary cortical senescence, we observed that the autophagic flux is impaired. The senescent phenotype is characterized by an increase of senescence-associated β-galactosidase activity, accumulation of DNA damage and activation of DNA damage response, accumulation of lipofuscin, increased levels of cell cycle inhibitors like p16/INK4A and p21/CDKN1A and the development of a senescent associated-secretory phenotype (SASP) which in turn propagates paracrine senescence and induces inflammation. In this work we aim to validate *in vivo* whether age-related autophagy changes correlates with the establishment of neuronal and glial senescence in mammalian aging brains.

**Keywords** : Aging brain; autophagy; cellular senescence; mammals

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# "Analysis of the 5-HT1A Receptor in Major Depressive Disorder in rat"

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In recent years, Major Depressive Disorder has increased alarmingly mainly affecting young people between 15 and 24 years. Several lines of research have been developed, which stand four important theories: immune, oxidative/nitrosative stress, imbalance of the hypothalamus-pituitary-adrenal axis and the deregulation of neurotransmitters, especially serotonin and their receptors. The aim of this work was to analyze if there are variations in the expression of the 5HT1A receptor in the hippocampus, using the OBX model. Male Wistar rats weighing 250-300g were divided into six groups as follows: control(C), surgery(S), OBX, OBX administered with vehicle (OBX+V) and OBX administered with antidepressant (OBX+A). Three weeks after surgery, behavioral tests were performed [avoidance to light test and analysis of motor behavior in the open field test(OFT)]. Antidepressant was administrated by period 21 days all days. All animals were sacrificed at the end of behavioral procedure and craniotomy was carry out to include in the analysis only animals with bilateral OBX without injury in other areas of the brain. The hippocampus was obtained for the analysis of 5HT1A receptor, AMP cyclic and Akt Bilateral OBX surgeries in the rat have been successful and OBX rats have decreased weight compared to surgery and control without extraction groups. The bilateral OBX surgery showed behavior of hyperactivity, and reduction in the grooming can be equated a symptom of depression in humans with respect to controls, Fluoxetine reduce these behaviors. In the smell stimulus recognition test the groups (OBX + V), (OBX + A) and (OBX), they showed the same trend in relation to the time of recognition to stimulus compared to surgery and control group these relation was maintained at the end of experiment. Fluoxetine reduces Akt factor which was increased by the action of the OBX, the 5HT1A receptor does not change during this process, although it should be noted that this may be due a decrease of neurons in the analyzed area, although there is a tendency to increase. This work, suggests that the OBX model is suitable to simulate the TDM, and that treatment with Fluoxetine for three weeks, does not improve all depressive mechanisms, which motivates us to propose new experimental tests to improve work realized on the receptor (5-HT1A), in major depressive disorder.

# Sigma 1 Receptor as a novel regulator of the TRPV1 ion channel

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Cell detects harmful environmental signals through protein receptors localized on the cell surface. Among these receptors is the TRPV1 ion channel which constitutes an important component of sensory system; this ion channel participates in the detection of thermal, mechanical and chemosensory stimulus. Furthermore, TRPV1 ion channel is an important therapeutic target for the treatment of chronic pain conditions such as diabetic neuropathy, arthritis and cancer.

Until now, there are few information about the regulation of TRPV1 expression and its interaction with other proteins. Recently, it has been related the pain mediated by TRPV1 ion channel with the protein named Sigma 1 Receptor (σ1R), a chaperone resident in the endoplasmic reticulum which it is regulated by different ligands.

Here, we used pain animal model, electrophysiological, molecular and cellular biology techniques in order to explore if σ1R regulate to TRPV1 ion channel. Cells transiently expressing rat or human TRPV1 ion channel incubated with the σ1R antagonist (BD1063) showed a decrease on the levels of total and cell surface TRPV1 protein. In accord with our biochemical assays, electrophysiological experiments confirmed a decreased on current-density evocated by TRPV1 activation. Moreover, the dorsal root ganglia (native TRPV1 expression system) from mice treated with the σ1R antagonist also show clearly decrease of TRPV1 total protein levels. We found that σ1R antagonism induced the degradation of TRPV1 protein through the proteasome pathway. Finally, in a pain animal model we found that σ1R antagonism decrease the pain induced by TRPV1 activation.

Our results highlight a mechanism involving σ1R on the regulation of TRPV1 protein levels and its role on the transduction on pain signal through the TRPV1 ion channel.

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# Effect of chronic Krill oil diet on epileptiform activity induced in adult rats by Pentylenetetrazole and with febrile seizures at 5 postnatal days.

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Epilepsy is a disease characterized by an enduring predisposition to generate epileptic seizures and by the neurobiological consequences of this condition. Epilepsy affects some 67 million people around the world and 80% of the patients live in developing countries. For that reason, it is important investigate new treatment options. Krill oil is an extract of Antarctic Krill, *Euphausia superba* and is one of the best sources of polyunsaturated fatty acids (PUFAS), in particular omega-3 and antioxidants, showing a neuroprotective role. In this work we evaluated the anticonvulsive effect of krill oil in adult rats with seizures induced by pentylenetetrazole and with febrile seizures induced at 5 postnatal days (PD). For this purpose, Wistar rats were EEG registered with superficial electrodes and were distributed in three groups: control (water, 1 ml, n=6, *****intragastric administration starting at birth and until 40-60 PD), palm oil (300mg/kg, n=5, intragastric administration*****) and krill oil (300mg/kg, n=6, intragastric administration*****). These animals had febrile seizures induced by hyperthermia at 4-5 PD. Each group was recorded 30 minutes (basal EEG activity), then animals of all groups received pentylenetetrazole (PTZ, 90 mg/kg i.p.; n=17, approximately 40-60 PD) in order to induce generalized seizures, and they were recorded for 3 more hours –if survived- after PTZ administration. The basal EEG of all groups showed high amplitude (between 170 and 338 μV) and low frequency (between 0.5 and 0.8Hz). The control group with water had a mean survival time of 19.6 min post-PTZ and the epileptiform activity showed a high amplitude (327.7±177.7 μV) and frequency (2.97±1.8 Hz). The control group with palm oil had a mean survival time of 24.7 min post PTZ and the epileptiform activity showed a high amplitude (398±248 μV) and frequency (3.29±2.82 Hz). The experimental group with Krill oil had a survival time of 52.1 min post-PTZ and the epileptiform activity showed a high amplitude (300±260 μV) and frequency (4.06±3.94 Hz). There were not significantly differences in theses parameters as well as in the number and duration of discharge trains between groups. In conclusion, we did not found any significant difference in animals from different groups in the analysis of EEG recordings.

El trabajo corresponde a la especialidad de Neurociencias, Epilepsia.

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# Isoforms of REST in vertebrados evolutionarily distant vertebrates

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**ABSTRACT**

REST is a zinc finger protein considered a neurogenesis master regulator in mammals. The tissue-specific levels of REST alterations usually trigger cancerous processes. So far it is unknown whether the evolutionary origin of REST took place in organisms with rudimentary nervous systems, and does not have a prototype of the superfamily of zinc finger proteins that could be the molecular ancestor of REST. Our *in silico* analyses show high conservation of REST *DNA Binding Domain* (DBD) and low conservation in *Represor Domain 1* (RD1), whereas te Represor Domain 2 (RD2) appears later in mammals. In our interpretation, the origin of a rudimentary REST capable to contact DNA, dates back to a time prior to the emergence of modern fish (500 m. A.), while the represor activity of modern isoforms were acquired during the onset of reptiles and specialyzed in mammals. We identified experimentally various isoforms of REST in extracts of skeletal muscle tissue of vertebrate organisms. Western blot and Immunoprecipitation results showed an isoform of 150-250 kD in *Iguana iguana*, *Streptopelia decaocto* and *Peromyscus mexicanus*, which matches the weight of the canonical isoform. Interestingly two bands of 71 and 37 kDa are present in all organisms studied. In addition, in *P. mexicanus* a variety of short isoforms were found, suggesting that new isoforms were acquired in vertebrates approximately 200 m.y.a. This work is the first experimental evidence in finding REST isoforms in evolutionarily distant animals and represents an initial step in the reconstruction of the natural history of this protein.

**Keywords:** REST isoforms, Evolution, Nervous System.

# Effect of neonatal administration clomipramine (CMI) on expression of REα and REβ in dorsal raphe nucleus

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Currently, depression is one of the most common and disabling mental illness, is a serious health problem worldwide. Neonatal treatment with clomipramine (CMI) in rats has been regarded as a model of depression as an adult it produces changes that resemble signs of depression in humans. These changes have been attributed to deficiencies in neurotransmission systems as; serotonin caused by treatment at critical stages of development. Among the alterations in the serotonergic system we are decreased levels of serotonin (5-HT) in limbic areas (like raphe nucleus), increased immobility behavior accompanied by decrease in swimming in the forced swimming test (FST). Currently it is proposed that hormones such as estrogen may be involved in the regulation of mood through the serotonergic system modulation. The aim of this study was to analyze the effect of neonatal administration of CMI on the expression of estrogen receptor (ER) α and β in raphe nucleus (production site 5-HT). For which Wistar rats were administered saline or CMI neonatally (8-21 days old) were used. Three months later they were divided into two groups for analysis of expression REα and REβ; 1) Immunohistochemistry and 2) qRT-PCR. To perform immunohistochemistry individuals were perfused and fixed and then dissect the brain and perform immunohistochemical technique. The second group were euthanized by decapitation and the raphe nucleus was dissected and then perform the analysis of mRNA expression for REα and REβ by qRT-PCR. Our results indicate that CMI treatment induces a decrease in expression of REβ in dorsal raphe nucleus indicating that treatment with CMI could cause alterations in both neurotransmission systems and their relationship to these with the endocrine system.

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# Evaluation of *Lopezia racemosa* extract with anti-inflammatory activity in a mouse model for the study of Alzheimer's disease

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Alzheimer´s disease (AD), is the main cause of dementia at a global level and occurs mainly in elderly individuals, the typical symptom of this pathology is the loss of memory. Evidence suggests that the accumulation of senile plaques formed by the aggregation of the peptide βA and tangles formed by tau protein affect the proper functioning of the central nervous system. AD studies are based mainly on animal models, such as those with rodents. Studies carried out with mice as a model of study, have evaluated the activity with background neuroprotective and anti-inflammatory extracts of plants, observing a decrease of damage at histological and memory level. There are reports of studies using double (PPP/Tau), or triple (PPP/Tau/ApoE) transgenic or induced (intracranial injection of the β-amyloid peptide) models to generate the characteristic features of the sickness, at both the cognitive and the histological level, with the purpose of improving the understanding and search for strategies that will help to ameliorate the symptoms or stop the progressivity of the disease. There is currently no cure for AD, only medications that decrease the manifestations, but with undesired side effects. Therefore, extracts derived from plants, are now alternatives used for the development of new drugs, as well as their potential for the relief of this illness. Thus the search for new natural alternatives which reduce the ailments and unwanted effects caused by drugs is of vital importance, as well as to keep proposing models that help study and improve the comprehension of this disorder. Hence the principal objective of this work is to evaluate the neuroprotective effect of a natural extract from *L. racemosa* in an induced AD murine model. To achieve this, a model will be established beforehand, consisting of intracranial injection of the peptide $\beta A_{42}$ in the CA1 region of the hippocampus of CD1 mice. Loss of spatial memory will be evaluated using the Morris Water Maze (MWM); furthermore, the presence of senile plaques and inflammatory response will be evaluated at the immunohistochemical (IHC) level using specific antibodies. To assess the neuroprotective effects of *L. racemosa,* mice will be divided in 4 groups. The first group (α group) will be AD induced by means of intracranial injection of $\beta A_{42}$ peptide, then will be administered intraperitoneally (i.p.) once a day for 30 days with extracts of *L. racemosa,* known to have anti-inflammatory properties. The second group will be AD induced, then i.p. injected daily with the drug tramiposate as positive control. A third group will be AD induced and then injected for a month with acetone (vehicle) as negative control group. Finally, a fourth group will be injected acetone i.p. once a day for 30 days without the AD induction (δ group). MWM will also be evaluated and corroborated at IHC level for each group.

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# Effect of a Synthetic Hypothalamic Hormone on Body Composition and mRNA Expression of Ghrelin and Lipoproteinlipase

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**INTRODUCTION:** Hypothalamic hormone agonist (HHA) has been reported for having direct effect on LH and FSH secretion, causing changes in body weight in experimental and clinical trials; however, no studies have been reported focused directly on body composition analysis and possible mechanisms involved. Ghrelin is an orexigenic peptide while lipoproteinlipase (LPL) an enzyme that can facilitate fatty acid storage in muscle and adipose tissues. mRNA expression of both components could be modified by HHA action.
**AIM:** To evaluate body composition and mRNA expression of ghrelin and lipoproteinlipase treated with HHA in female rats

**METHODS:** Female Wistar rats (146.8 g ± 3.8) were used, two groups were formed as follows: a non-ovariectomized group (HA) and ovariectomized group (OVX+HA) both treated with a HHA dose every 72 hours for 120 days (5 µg/Kg). Likewise, another non-ovariectomized (CTRL) and one ovariectomized (OVX) groups were formed, they only received an equivalent volume of vehicle (0.9% NaCl). Every 20 days body composition was assessed using hydrodensitometry techniques, carcass analysis and chemical analysis to determine fat mass under skin. Additionally, RT-PCR was performed to measure the mRNA expression of ghrelin and lipoprotein lipase.

**RESULTS:** It was found that OVX group had an increase in weight by 51% while the HA group by 47%, OVX+HA and CTRL 38% and 33%, respectively compared to baseline. The analysis of fat mass (FM) was 14% higher for HA vs CTRL and 19% higher for OVX vs OVX+HA, fat-free mass (FFM) was higher in the HA group compared to CTRL while OVX and OVX+AH showed a pattern very similar for both components. At the end of the experiment the mRNA expression of ghrelin in the HA group was 75% higher than OVX+HA while CTRL group had an expression of 25% lower compared to OVX. The LPL mRNA expression was 48% higher for HA compared to OVX+AH group. Interestingly CTRL group had an expression of 51% lower compared to the OVX group.

**CONCLUSION:** The HHA promotes changes in weight and therefore in body composition also generates significant changes in mRNA expression of LPL and ghrelin which means that mechanisms of orexigenic signaling and fatty acid storage maybe are implied. Also the results indicate a direct effect of HHA regardless of the presence or absence of gonadal hormones on body weight and composition.

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# Novel regulators of TRPV1 ion channel: effects in pain and itch sensations

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Pain and itch sensations are encoded and transmitted by primary sensory neurons (nociceptive neurons and pruriceptive neurons, respectively) in the trigeminal ganglia (TG) and dorsal root ganglia (DRG). These neurons mainly expressing the TRPV1 ion channel which is a receptor widely associated to detect noxious thermal, chemical and physical stimulus. Moreover, endogenous molecules, such as LPA (lysophosphatidic acid) and histamine directly or indirectly, respectively, activate to TRPV1 ion channel.

Data from our laboratory have shown the potential of certain bioactive phospholipids and omega-9 fatty acids as regulators of TRPV1 activation, which opens a field to determinate the physiological role of these compounds.

Here, the assessment of pain and itch was rendered in different animal models to evaluate the physiological role of cPA and omega-9 fatty acid in TRPV1 regulation. C57BL/6J mice were used for paw-licking assay and neck model in order to determine the effects of cPA injection. We found that cPA is a mediator of pain and itch sensations through TRPV1 activation, these data were supported by results obtained from mouse lacking of TRPV1 expression (Trpv1- / -) wherein pain and itch were considerably reduced.

We also evaluated itch caused by histamine injection through neck and cheek model experiments. Our results confirmed the pruritogenic role of histamine through the indirect TRPV1 activation. Moreover, we found that an omega-9 fatty acid was able to reduce the itch caused by histamine and cPA and also decrease the pain induce for the TRPV1 activation by capsaicin and LPA.

Our results clearly show the potential of an omega-9 fatty acid for relieving pain and itch mediated by the activation of the TRPV1 ion channel.

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# Kinetics of serum proinflammatory cytokines in a murine model of Alzheimer´s disease

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Amyloid- β (Aβ) is a peptide fragment generated from an abnormally cleaved Amyloid Precursor Protein (APP). The most common length of the peptide is constituted by amino acid residues: (1-40; 1-42; 25-35) of which only the 1-40 fragment is non toxic. The other two fragments share immunoreactive characteristics and have toxic activity (necrosis, cellular stress, destruction of tight junctions). The presence of Aβ (1-42) deposits and Aβ (25-35)(senile plaques), in the hippocampus, a histopathological characteristic of Alzheimer's disease (AD), is accompanied by oxidative stress, changes in proinflammatory serum Aß (1-42) and interleukin concentrations. However, AD diagnosis is presumptive and a confirmatory post-mortem autopsy is required to confirm the diagnosis. Unfortunately the initiation and progress of the disease, both at the brain and systemic level, is difficult to determine with precision. Therefore, experimental animal models to understand the development of AD have been developed; one of this model consists in the injection in the hippocampus of Aß (25-35) in rats. However, it has never been determined if the Aß (25-35) peptide is able to induce systemic oxidative stress and immune reactions. Our aim was to determine if hippocampal administration of Aβ(25-35) in Wistar rats could induce a systemic modification in serum inflammatory cytokines and oxidative stress detectable in serum. Nitric Oxide, IFN-γ, TNF-α, IL-6, IL-10 and IL-17A were determined in serum of Wistar rats at different times after hippocampal injection of Aβ (25-35). The results showed an increase in nitrites concentrations in the hippocampus after 1.5 hours and increased levels of pro-inflammatory cytokines 3 hours after Aβ injection followed by a systemic anti-inflammatory response 14 days after Aβ administration. The presence of Aβ in hippocampus generates an acute systemic pro-oxidant and pro-inflammatory response that wanes after 14 days.

# Analysis of cytoskeletal proteins from spinal cord neurons of rat embryo treated with gonadotropin releasing hormone (GnRH).

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It is widely recognized the role of GnRH in the reproductive process; inducing the synthesis and secretion of pituitary hormones such as follicle stimulating and luteinizing. However, recently it has been described that administration of GnRH induces an increase in axonal diameter and cytoskeletal protein expression in the spinal cord of rats with spinal cord injury. The aim of this study was to analyze the expression of cytoskeletal proteins, neurofilaments of 68 and 200 kDa, and as a marker of the synaptogenesis spinophilin in spinal cord neurons in culture. Wistar rat embryos of 15 days of gestation as donors of spinal cord neurons were used, which were dispersed by mechanical-enzymatic technique. Neurons were seeded at $5X10^5$ in DMEM culture medium and maintained in incubation with carbogen at 37° C until use. For neurofilament of 68 and 200 kDa analysis, neurons were incubated for 24 hours with 100 nM of GnRH and expression was analyzed by western blot and for constitutive expression β-actina was used. For spinohilin expression analysis, neurons were incubated at different times (4, 24 and 72 h) and concentrations (0.1, 1, 10, 100 and 1000 nM) and mRNA expression was studied by PCR and for constitutive expression GAPDH was used. The results show that incubation with GnRH induces a significant increase in the expression of neurofilament both 68 (18%) and 200 kDa (10%), as well as spinophlin (25%). In conclusión, both cytoskeletal proteins neurofilament as spinophilin, increased after treatment with GnRH in cultured neurons of spinal cord of rat embryo; which could mean an increase in both axonal diameter, and the number of interneuron synaptic contacts with possible effects of neuroplasticity.

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# Abnormal expression of matrix metalloproteases 9 and 2 in individuals with Alzheimer's disease

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**Introduction**: Alzheimer´s disease (AD) is a progressive neurodegenerative disorder and the major cause of dementia. The main pathological hallmarkers include senile plaques (deposits of improperly processed β-amyloid (βA)), neurofibrillary tangles (aggregates primarily composed by protein Tau), as well as neuronal death and synapses loss. Matrix metalloproteases (MMPs) are $Zn^{2+}$ and $Ca^{2+}$ dependent endopeptidases which can degrade components of the extracellular matrix. Previous studies have shown that MMPs are able to cleave the amyloid precursor protein, βA and Tau; further studies observed that the Tau cleavage by MMP-9 improves the Tau oligomer formation. Recent studies have found an increase of MMP-2 expression in astrocytes surrounding senile plaques in transgenic mice brain. Some data indicate that deregulation of MMPs can also occur at early stages of AD. **Objectives**: To compare the levels of MMP-9 and MMP-2 between individuals at different stages of AD and controls. **Materials and Methods**: The tissue was crushed, sonicated and centrifuged with homogenization buffer and protease inhibitors. The quantification of proteins was carried out by Microbradford. The western blotting was performed using 60μg of protein and resolved in SDS-PAGE using 10% separing gels followed by transfer to nictrocellulose membranes and incubated with anti-MMP2 and anti-MMP9 antibodies and reveled with secondary antibody conjugated with HRP. Zimography was performed using 30μg of protein using 8% polyacrylamide gels copolymerized with gelatin 1mg/ml. The gels were incubated with activation buffer and stained with Coomassie. For Immunohistochemistry, the tissues were treated with citrates buffer, peroxide, blocked, incubated with respective antibodies, treated with DAB, and counter stained with hematoxilin. For confocal microscopy, the tissues were stained with Sudan black, blocked and incubated with anti-MMP2 and anti-MMP9 antibodies. TG3, Tau 423, Tau C-terminal were used as markers of the stage of AD. **Results and Conclusions**: The levels of expression of MMP-2 and MMP-9 in tissue from cases of AD were increased compared with controls. The intermediate stages of AD presented highest levels of MMPs and were found near Tau lesions. These findings suggest that the MMPs have a major role in the evolution of the disease.

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# Role of enteric glia in mesenteric afferent nerve activity

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The enteric nervous system consists of ganglia and fibers enclosed in the walls of the gastrointestinal tract, it coordinates and regulates different functions like: motility, digestion and secretion in synchrony with the central nervous system. The enteric ganglia consist of neurons and glial cells; these outnumber neurons and are found in both the myenteric and submucosal plexuses, embrace nerve fibers and varicose release sites within enteric ganglia and appear to participate in maintaining the homeostasis of neurons. Little is known about the physiological role of glial cells in the enteric nervous system. Previous studies have shown that the glial cell disruption by a gliotoxin (fluoroacetate) reduce gastrointestinal motility in animals. However, there are not reports on their role in the mesenteric afferent nerve functions. The aim of the study was to elucidate the role of enteric glia in the sensorial activity of the gut and in the multiunit afferent activity in mesenteric nerves, both spontaneous and induced by increasing intraluminal intestinal pressure. It was carried out the dissection of a segment of jejunum C57BL-6 mouse intestine; a nerve was dissected from the mesenteric border, which one multiunit afferent activity was recorded. The spontaneous action potentials in response to the application of intraluminal pressure were recorded. Fluoroacetate was applied by extraluminal perfusion (3-5 mM during 5-10 min); this decreases afferent response to intraluminal pressure progressively. The maximum effect was observed around 50 minutes, time in which the response of the high threshold fibers is almost inhibited and the low threshold fibers decrease to one third of the control value. In addition multiunit basal activity remains unchanged. The results suggest a relevant role of enteric glia in mesenteric afferent nerve activity. The fact that the spontaneous activity is unchanged suggests that participation would be on a sensory mechanisms that detect the tension of the intestinal wall.

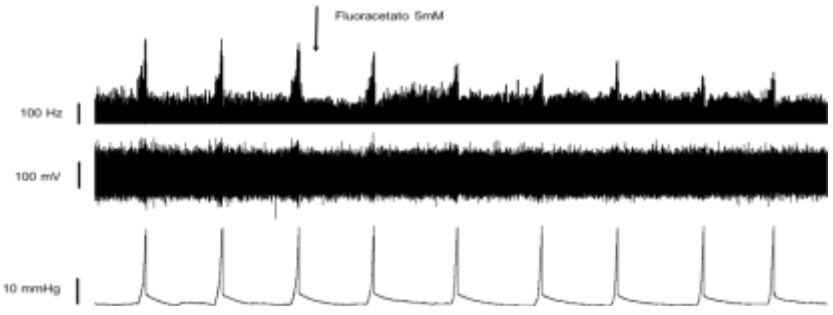


Fig 1. Frequency histogram and recording of a multiunit mesenteric nerve activity (Spontaneous and induced by increases of intraluminal pressure)

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# STIM and Orai as molecular biomarkers in calcium regulation in Alzheimer's disease.

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Alzheimer's disease (AE) is a neurodegenerative central nervous system (CNS) characterized by progressive and irreversible neuronal loss. Alzheimer histologically characterized by two structural changes, neuritic plaques and tangles neurofibrales. Its high rate of prevalence is the fourth leading cause of death (WHO. 2012). There are currently 35.6 million sufferers in the world, Mexico 350,000 cases of which 2030 are reported annually die. Despite his extensive research is still unclear etiopathogenesis limiting the development of efficient drugs and timely diagnosis. This work is based on the hypothesis of calcium which states that brain atrophy is caused by alterations in calcium homeostasis and this, a key process in the pathogenesis of the disease. Calcium ion channels that were studied in this work are STIM1 and Orai1, in order to analyze their participation in the AE.

*In silico* and Orai1 STIM1 proteins in order to know their structure for molecular modeling of these proteins analyzes were performed. In silico analyzes in suggest that calcium homeostasis regulated by Orai1 STIM1 and ion channels could be crucial in the beginning and during the disease and could be used as potential molecular biomarkers for disease detection.

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# Role of reactive oxygen species in palmitic acid-induced neuronal insulin resistance

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The intake of high fat diets (HFDs) contributes to the development of metabolic alterations such as insulin resistance, obesity and type II diabetes mellitus. In the central nervous system (CNS), the HFDs generate structural and functional changes associated to insulin signaling reduction. Palmitic acid (PA) is the most abundant fatty acid in the HFDs and has been found to play an important role in the development of insulin resistance in hypothalamus, liver, pancreas and skeletal muscle. Cellular PA metabolism increases ROS, reduces the cellular content of NAD+ and increases ceramide production. Insulin regulates several aspects of neuronal function such as neuronal differentiation, survival and plasticity. It has been described that an essential part of the insulin signaling is the generation of a pulse of hydrogen peroxide ($H_2O_2$) produced in response to the binding of insulin to its receptor. Therefore the presence of reactive oxygen species (ROS) by pathological periods and concentrations may have an inhibitory effect on insulin signaling. It is still unknown if PA also participates in the development of neuronal insulin resistance neither the mechanism involved. Thus in the present study we have evaluated the role of PA on inhibiting neuronal insulin effects and the participation of ROS. We used human neuroblastoma cells (MSN) differentiated to neurons with retinoic acid and nerve growth factor. Neurons were exposed to PA (200μM) for 1 h and then we measured metabolic activity by the MTT reduction method. We found that insulin (10 μM) increased MTT reduction by approximately 20% as compare to control neurons. The insulin-induced metabolic activation was totally prevented by PA exposure. We also found that PA impeded the phosphorylation of Akt (S473) in response to insulin, suggesting the development of an insulin resistance state. The incubation with the mitochondrial antioxidant, MitoTEMPO, prevented the inhibitory effect of PA on insulin metabolic activation. At present these results point to the participation of PA in reducing neuronal insulin signaling as well as the role of ROS production in such effect.

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# Time-restricted feeding exerts anti-inflammatory and neuroprotective effects on acute seizure model.

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## Summary

**Introduction:** During the past decade, experimental research has demonstrated a prominent role of the pro-inflammatory molecules on epileptogenic and ictogenic processes. On the other hand, our research group recently demonstrated that time-restricted feeding (TRF) had an anticonvulsant effect, however, the precise mechanism by which this diet exerts its beneficial effects are still unknown. **Objective**: Our aim was to investigate whether TRF is able to exert its beneficial effect decreasing the expression of pro-inflammatory molecules and thus might have a neuroprotective role after seizure induction. **Methodology**: Briefly, TRF consisted in allowing rats to eat for two hours daily during their light phase for 20 days; conversely, control group was fed *ad libitum* (AL). After dietary schedule, *status epilepticus* (SE) was induced using a pre-treatment with a injection of lithium chloride (3 mEq/kg) followed by pilocarpine administration (60 mg/kg). Both protein and mRNA expression of pro-inflammatory molecules such as interleukin 1 beta (IL-β), tumor necrosis factor alpha (TNF-$\alpha$) and interleukin 6 (IL-6) were measured in hippocampus from each group 24 h after SE. Additionally, coronal brain slices were processed with fluoro-Jade C to mark degenerate neurons. **Results:** Our preliminary data showed that the group that followed TRF before SE had a significant decrease in both of mRNA and protein expression of pro-inflammatory molecules (IL-β, TNF-$\alpha$, IL-6), in comparison with AL group after seizure induction. Furthermore, the hippocampus from TRF group showed a significant decrease on reactive gliosis and less FluroJade-positive cells were observed after SE. **Conclusion:** Our data demonstrate that TRF may exert a neuroprotective effect by decreasing the mRNA and protein expression of pro-inflammatory molecules and reactive gliosis after seizure induction.

**Key words:** Time-restricted feeding, status epilepticus, cytokines, IL-β, IL-6, TNF-α.

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# Effect of Taurine in the Progenitor-like Cell Generation from Müller Glia of mice.

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A variety of diseases induce retinal neurodegeneration, leading to irreversible blindness. However, no tratments are available to halt neurodegeneration completely or enable regeneration and reestablishment of retinal fucntions afer retinal injury. In the last two decades, Müller glia (MG) have been considered as a source of stem cells of retinal regeneration in different species, including primates and humans. Until now, it has been observed the exression of neurogenic genes and signaling events, that induce MG cells to display properties of retinal progenitors in the injured mammalian retinas. Along with the identification of signaling pathways, a number of molecules have also been implicated in the regulation of cell proliferation. One of these molecules is the taurine, a sulfo-containing amino acid present in high amount, often in the mM range, in most vertebrate species. Is not involved in metabolic reactions, excepted the taurocholate synthesis. Numerous studies have demostrate the key role of taurine in the functional and anatomical homeostasis of neural retina. In taurine-deficient experiments *in vivo* and *in vitro* shows defective migration and delay in cell prolifferation in mice, cats, monkeys and humans. In this work, we tested the effect of taurine in the induction processes of MG to generate progenitor-like cell in culture. MG progenitor-like cells were obtained from isolated mice retina (Post 9), and maintained in the progenitor-promoting medium with or without taurine. In pressence of taurine we observe an increment of MG progenitor-like cells, in a concentration-dependent manner. The maximal effect was found at 10 mM of taurine with a 53% of increase compared with the control, these effect was observed along the pasages (P1-P3). The trypan blue exclusión assay, indicate a moderate improvement in the viability of the cell exposed to taurine (15-20%). The BrdU incorporation pattern in the presence of taurine showed a rise of 45%. In presence of taurine, the proportion of MG progenitor-like cells positive to markers for neuronal and glial cells was more evident compare to the control. In conclusion, these exeriments indicate that taurine increased the number of MG progenitor-like cells and improve the proliferation range, suggesting a markedly influence in the magnitud and time curse of DNA synthesis. The mechanism(s) of the stimulatory effect of taurine on cell proliferation here described are still unknown. On the other hand, these results sugest that, under the right conditions, MG might be induced to adopt characteristics of a retinal progenitor that could be used for retinal neurons repair.

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# Gene expression of metabotropic glutamate receptors in the vestibular system of chicken embryo

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**Abstract.**

The purpose of this work was to identify the presence of metabotropic glutamate receptor (mGluRs) during embryonic development of the vestibular organ of the chicken (Gallus domesticus). We performed a RT-PCR assay in order to assess levels of expression of the mRNA encoding the different subunits of metabotropic glutamate receptors, both crista ampullaris and vestibular ganglion. This study will allow us to establish the existence of a pattern of expression of the different subunits, depending on the age of the chick embryo (E14, E16, E19 and E21). For each experimental series 108 crests and 36 ganglions from chick embryos. Were used total RNA from crista ampullaris and vestibular ganglion was isolated using Quick-RNATM MiniPrep, (Zymo Research, USA) according to the instructions provided by the manufacturer. The concentration of total RNA for each sample was determined by absorbance measurements at 260 and 280 nm (Biophotometer, Eppendorf, USA.). cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA). cDNA obtained was subjected to the PCR protocol. To verify their identity, amplification products were resolved by electrophoresis in 1.5% agarose gels and visualized by ethidium bromide staining, and the intensities were then measured by scanning the gel with the ChemiDoc Gel Documentation System (Bio-Rad, USA). RT-PCR experiments revealed the presence of the three receptor groups. mGluRI and mGluRII increases its presence at ages near hatching, while mGluRIII has the most important early stage expression (E16). Our results suggest the presence of metabotropic glutamate receptors in vestibular afferent synapses of the chicken during embryonic development.

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# EFFECT OF PROLACTIN ON MICROGLIAL ACTIVATION AND EXPRESSION OF PROINFLAMMATORY CYTOKINES IN THE HIPPOCAMPUS OF MALE RAT PUPS

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Abnormal activation of hormones and neurochemical systems early in life causes a high vulnerability to a number of physiological, behavioral and psychiatric disorders long term. It was observed that daily administration of the hormone prolactin (PRL) 13 mg / kg of body weight to neonatal rats (postnatal days PND 1 to 14) decreases significantly neurogenesis of hippocampal hilus at PND 15 and triggers a depressive like behavior at adult stages. The mechanism by which this hormone exerts these effects is unknown; one possibility is that PRL acts as a cytokine altering the immune response of the central nervous system. The neuroimmune system is constituted by glial cells divided in microglial and astrocytic cells; these are distributed throughout the brain and can be activated by infections, inflammation, and stress, altering the expression and the secretion of cytokines. The aim of this study is to analyze the effect of PRL on microglial and astrocytic cells in the hippocampus by comparing the cytokine expression in the hippocampus and the peripheral response of proinflammatory cytokines, under basal conditions or after stress exposure, on postnatal PN day 15. Six groups of male Sprague Dawley pups (n = 6 for each group) were randomly divided in the following groups: 1) control-basal 2) Control-stress, 3) vehicle-basal, 4 ) vehicle-stress 5) PRL-basal 6) PRL- stress Control groups were left undisturbed at the mothers nest, the vehicle groups were injected with saline solution and the PRL groups were injected with a dose of PRL 13mg / kg body weight daily from PN1-PN14, at PN15 the pups were sacrificed under basal conditions or after subjecting them to a stressor ( 3h maternal separation) before sacrifice. Afterwards the hippocampus was obtained and blood samples were collected from animals. Concentrations of proinflammatory cytokines (IL-6, IL-1β, TNF) in plasma were determined using the ELISA technique. The mRNA expression of proinflammatory cytokines in hippocampal extracts was analyzed by qPCR. The mRNA expression of IL-6, IL-1β, TNF increased in vehicle-stress pups but were decreased in the PRL-stress group. The peripheral concentration of IL-1β decreased, TNF a increased, and the concentrations of IL-6 did not change in PRL stress pups. In conclusion PRL decreases the expression of proinflammatory cytokines in the hippocampus, but PRL causes an independent response of peripheral cytokines. These specific responses, together could reduce hippocampal neurogenesis and contribute to the alteration of the neuroendocrine axis.

# Evaluation Of The Anti-inflammatory Capacity Of Methanol Extracts Of Wild Plants In Southern Sonora.

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Rheumatoid arthritis (RA) is the most common autoinflammatory disease that affect 0.5-1 % of adults in the world. Uncontrolled cases of RA causes joint damage, pain, chronic inflammation, disability, decreasing life quality, and other comorbidities. Although some environmental factors are associated with RA, 50 % of the risk is attributed to genetic factors, which complicates its treatment. The clinical anti-inflammation clinical therapies are unsatisfactory, thus the search for new drugs is needed. At the present, ethnobotanical knowledge has played an important role finding alternative treatments, and many studies have shown anti-inflammatory properties of some medicinal plants. In Sonora, the herbal formula from Traditional Medicine is widespread in the population as a treatment for diseases like cancer, diabetes, arthritis and inflammatory diseases. This work focused on evaluating the anti-inflammatory activity of various regional plants by determining the genetic expression of TNF-α by RT-PCR in macrophage cultures stimulated with LPS.

Keywords: Medicinal plants, Anti-inflammatory

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# *In vivo* PARAMETERS IN A MODEL OF TYPE 2 DIABETES MELLITUS IN RATS TREATED WITH PIOGLITAZONE

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Introduction. Type 2 Diabetes Mellitus (T2DM) is a disease caused by a progressive defect in insulin action and a chronic state of hyperglycemia. Insulin resistance is central to the pathogenesis of the disease. It is important to use reliable models in order to establish new therapeutic alternatives for the treatment of T2DM. The aim of this study was to standardize a T2DM model in rats with the administration of a single dose of streptozotocin (STZ) and pioglitazone (PIO) at an oral dose of 10 mg/kg/day for 15 days.

Methodology. 53 male Wistar rats weighing 240 ± 20 g were used. Four groups were formed (n= 6 animals each group): Control, Vehicle of T2DM, T2DM and T2DM+PIO. TDM2 model was generated by a single dose of 45 mg/kg STZ intraperitoneally (i.p.) and 15 rats were not affected. Fasting blood glucose and body weight were recorded weekly. Glucose tolerance tests (GTT) with anhydrous glucose (2 g/kg, i.p.) solution were performed weekly for 4 weeks. PIO (10 mg/kg/day) was administrated for 15 days (weeks 4 and 5). A commercial glucometer (Accu-check; Roche) was used. Data were statistically analysed by univariate analysis of variance (ANOVA) and mean values were compared with Dunnett's test.

Results. Control group (group I) presented a continuous and a total weight gain of 100%. The vehicle DM2 group (group II) had a similar gain (67%). Significant difference ($p < 0.05$) was found in T2DM group (group III) and treatment of diabetic animals with PIO (Group IV). Those had a lower weight gain (31% and 27% respectively) after administration of STZ. In Fasting blood glucose there was a significant difference between group I and groups III and IV, but not with group II. In addition, administration of PIO (10 mg/kg/day) resulted in a significant reduction of glycemia in group IV. The shape of the curves obtained by Glucose tolerance tests (GTT) were very similar between groups I and II. This contrasted with groups III and IV, since they exhibited significant difference. At the last week, there was significant difference at minute 60 between groups III and IV.T2DM model results are contrasting with group I. An increase in blood glucose and low weight gain was observed. The decrease in body weight in diabetic rats may be due to the loss or degradation of structural proteins caused by the administration on SZT. T2DM group treated with PIO for 15 days (Weeks 3-5) had blood glucose levels similar to those of group I. Also, at GTT performed in week 5, there was rapid absorption of glucose but it had a tendency to normallize glycemia faster,while untreated diabetic animals shown a slower glucose absorption during the tests. It is suggested that PIO generated insulin sensitization.

Conclusions. In conclusion, an intraperitoneally STZ administration of 45 mg/kg developed a suitable T2DM rat model. Furthermore, a PIO dose of 10 mg/kg/day generated a decrease of glycemia.

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# Effect of a novel thiazolidinedione derived analogue on *in vitro* insulin-like response

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Diabetes mellitus (DM) affected 382 million people worldwide in 2013 and is estimated to increase to 592 million in 2030. In Mexico it is the leading cause of death in women and the second in men. Antidiabetic drugs in use have a number of adverse events such as gastrointestinal disturbances, hepatotoxicity and weight gain. Our working group synthesized a thiazolidinedione derived analog in order to improve their pharmacokinetic properties and decrease toxicity. The aim of this study was to determine the effect of the thiazolidinedione analog on adipogenesis and insulin-like response in 3T3-L1 preadipocytes. Nontoxic concentration of the thiazolidinedione derived analogue resulted in 20 μg/mL. Rosiglitazone (RGZ) was used as positive control at a concentration of 10 μg/mL. Adipogenesis was induced using DMEM supplemented with fetal bovine serum at 10%, 5 μL/100mL insulin, 0.5 mM 3-isobutyl-1-metyl-xantine and 0.4 μg/mL dexamethasone; the insulin resistance was induced with 10 ng/mL of the Tumor necrosis factor alpha (TNF-α). The insulin response was assessed by the accumulation of triglycerides determined colorimetrically or by incorporating a glucose analog 2-[N-(7-Nitrobenz-2oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose (2-NBDG) by fluorometry. No adipogenic effect on preadipocytes was observed by the thiazolidinedione analogue in the absence of insulin; however the compounds RGZ and thiazolidinedione derived analogue increased the insulin-like response in resistant adipocytes. Currently the possible mechanism of action of thiazolidinedione analog is studied.

**Palabras clave:** Adipogenesis, adipose differentiation, L-carnitine, insulin resistance, insulin-like response.

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# *CYP2C9*3* Genetic Variant Is Independently Associated with Glycemic Control in Type 2 Diabetes Patients Treated with Glibenclamide

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**Introduction**: In México, Type 2 Diabetes is the second cause of death due to lack of glycemic control leading to macro and microvascular complications. Only 30% of patients with diabetes achieve HbA1c ≤ 7%. Glibenclamide is a sulfonylurea, frequently used in the treatment of diabetes and is metabolized by CYP2C9. The aim of this study was to determine the association of *CYP2C9*2* and *CYP2C9*3* genetic variants with glycemic control in type 2 diabetes patients treated with glibenclamide.

**Method:** Case-control study of type 2 diabetes patients treated in the IMSS. Patients with time of disease up to 15 years and receiving treatment with glibenclamide or glibeclamide plus metformin, were included. Patients with HbA1c > 7% were considered cases, while patients with HbA1c ≤ 7% were considered controls. Genotyping was performed by real-time PCR using TaqMan probes for genetic variants CYP2C9*2 and CYP2C9*3. In bivariate analysis, OR were calculated with confidence intervals at 95% (CI95%). For the multivariate analysis, a multiple logistic regression that included major confounders was performed.

**Results**. Four hundred and fifty patients were included in the study. Median age of participants was 50 years (range 22-81) and median time to disease progression was 6 years (range 1-15). One hundred thirty-one patients were men (29.1%), 119 exercised regularly (26.4%) and 268 were treated with glibenclamide plus metformin (59.6%). No deviations were observed in Hardy-Weinberg equilibrium. In bivariate analysis, *CYP2C9*3* gene variant was associated with glycemic control (OR = 0.404, CI95% [0.171-0.956]), while the *CYP2C9*2* gene variant showed no association (OR = 1.041, CI95% [0.563-1.928]). In multivariate analysis, the *CYP2C9*3* gene variant retained its association (OR = 0.332, CI95% [0.135-0.813]) when adjusted for age, time of disease, physical activity, BMI and concomitant use of metformin.

**Conclusion**: *CYP2C9*3* genetic variant independently contributes to glycemic control in Patients with Type 2 Diabetes treated with glibenclamide.

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# Characterising the toxic activity profile of Caybdea marsupialis venom.

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Abstract

The cubozan *Carybdea marsupialis* is widespread in tropical waters and is well-known due to their stinging effects. Like other cnidarians its venom include different kinds of toxins which can be divided into three main categories: neurotoxic peptides, phospholipases and high molecular mass cytolytic pore forming proteins. Although the wide array of toxins from this organism are used fundamentally for hunting and defence, they have clinical importance owing to their effects in envenomed humans. Thus, the current research has been focused on the characterisation of venom effects and venom proteins isolation from a large number of venomous box jellyfishes, including *Carybdea marsupilais.* Unfortunately, despite the efforts only a few toxins have been isolated and characterised because of issues relating to protein instability, as well as, variability in protein resources, extraction methods and analytical techniques. Compared with other cnidarians, *C. marsupialis* has been slightly studied and only a few cytolycins (42-46 kDa) has been isolated form other members of cubozoa class. To date, accumulating evidence suggests that cubozoan jellyfish produce at least one group of homologous labile and basic bioactive proteins. This group of proteins are potentially lethal and due to their secondary structure analysis and protein homology prediction, it is suggested that this toxins may act as a pore forming toxins. This fact can explain some of the symptoms reported on envenomed humans like inflammation and necrosis, but cannot explain other reported biological activities related with neurotoxicity and the burning cutaneous pain sensation. In this work biological activities from three fractions from *C. marsupialis* venom extract were determined, suggesting the presence of $PA_2$ activity and neuropeptides.

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# A novel therapy for breast cancer triple negative mediated tumor suppression through mTOR inhibition and glycolysis aerobic

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Breast cancer is a public health concern, in Mexico it is the most common cancer in women, with 23 764 new diagnostics every year and 6 591 deaths in 2015. Breast cancer is an heterogeneous disease, composed of multiple subtypes, from which triple negative, or basal-like, breast cancer, stands out because of its high proliferation, poor prognosis, and lack of an effective therapy. Previous studies have shown that the breast cancer progression can be associated with deregulated cellular energetics (Warbung 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 novel pharmacological 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 breast cancer treatment. We estimated inhibitory concentration ($IC_{50}$) in individual and combination therapies. Our results showed that the triple therapy was less toxic than doxorubicin alone and that it suppressed the proliferation in an *in vivo* model. We next examined colony formation and found reduced activity in cells treated with the triple therapy. Finally we assessed cell death through flow cytometry; cells exposed to the triple therapy showed early apoptosis, while those treated with doxorubicin showed late apoptosis. Our results provide useful clues for targeting deregulated cellular energetics in triple negative breast cancer through metabolism inhibitors.

**Key words**. Breast cancer, Warbung effect, metformin, sodium oxamate and doxorubicin.

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# Molecular analysis of cell death type induced therapy coadyuvant: doxorubicin-metformin and sodium oxamate as inhibitors of the mTOR pathway and glycolysis cell model in a triple negative breast cancer.

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Triple Negative Breast Cancer (TNBC) is one of the leading causes of death among women around the world. Current therapies are aggressive and inefficient, generating several collateral effects in patients. In the urge to explore new therapeutic approaches, we propose an alternative treatment for breast cancer based on the correction of the tumoral cell metabolism leading to apoptosis and autophagy. Under this scheme, we tested the combination of three well known medicines -metformin, sodium oxamate and doxorubicin-, based on their mechanisms of action that affect different aspects of the metabolism of the tumoral cell. Previous studies in our working group; showed *in vitro* we found synergy between the three compounds in comparison to the positive control (doxorubicin) and in vivo Xenotransplant of MDA-MB-231 cells was performed on nude mice, development and progression of disease were followed by microPET with (18F)FDG uptake compared to doxorubicin. Treatment was started once the tumor size reached 3 $mm^3$. A complete remission of the tumor size was observed after eight days of treatment, no visible side effects were detected and no disease recurrence was presented during the remaining lifetime of mice.

The aim this work is analize the molecular level in BC MDA-MB-231, MDA-MB-468 and BT20 cells line. Inhibition mTOR protein as well as the enzyme LDHA, and furthermore, induction of autophagy process generated by activating LC3, a primordial protein implicated in the conformation and elongation of the autophagolysosome. The induction autophagy was also determined by electronic transmision microscopy (TEM) and green flourescent protein GFP-LC3; both of them assys showing a considerable detection of LC3. The detection apoptosis was determined trought release LDH-A by cytotox96 (Promega). All treatments were compared with doxorubicin. Our results bring relevant implications for breast cancer treatment, they provide evidence about the link of apoptosis and autophagy utilizing mTOR and glycolysis inhibitors.

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# Analysis of the presence of adducts of Benzo [a] pyrene in the DNA of placental tissue as a measure to environmental genotoxic compounds exposure during pregnancy.

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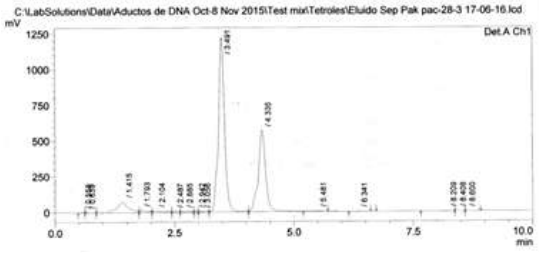
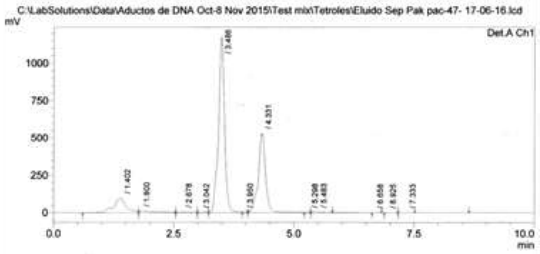
Department of Environmental Health Sciences and Epidemiology.Environmental pollution is an issue of global concern, due to the direct impact and may be specially critic during pregnancy. Compounds such as particulate matter (PM10 and PM2.5), which consist of a mixture of metal oxide aggregates suspended and organic materials such as Polycyclic Aromatic Hydrocarbons (PAH, Benzo [a] pyrene). Exposure to these contaminants may lead to adduct formation in the DNA. These alterations have been associated with the outcomes. The mechanisms by which prenatal PAH exposure might impair mental development are unknown but may result from decreased head circumference at birth and anti-estrogenic effects. Studies that attempt to associate prenatal exposure with childhood health problems post-birth[1] cannot always distinguish whether the pre- or postnatal PAH exposures are of most relevance, however. Mexico City experiences high ambient PAH levels compared to many world cities. [2,3] This suggests that, PAH exposures may play an important role in fetal development, and opportunities for prevention of adverse birth outcomes through reduction of emissions from vehicles and other air pollution sources are great. **The aim** of this work, is to document damage to the DNA by exposure to PAH using placental tissues as the source of the DNA and to correlate this with the outcome of pregnancy. **Material and methods.** Placental tissues obtained from the Mexico City's Perinatal Cohort were used for DNA extraction. After acid hydrolysis, the sample were concentrated and analyzed in a Shimadzu HPLC system with RF-10Axl spectrofluorometric detector, using a Shimadzu SIL-10A automatic sample injector to minimize batch effects[1,2,3]. Adduct concentration was calculated by comparing against the peak areas of an external calibration curve, generated from the fluorescence peak of an authentic BPDE tetrol standard, for every set of samples. Detection limits are approximately, 0.5 adducts/$10^{-8}$ nucleotides, meaning 0.5 pg /100 µg DNA. **Results**. At present, we have a bank of DNA samples (n = 200). Assays performed heretofore, enable demonstrate that the B[a]P-tetrols, released from human DNA, after acid hydrolysis and analyzed using reversed-phase HPLC corresponding to BPDE-DNA adducts present in the DNA. Representative profiles of the hydrolysis products from two different DNA samples (patients #29 and # 47), are illustrated in figure. The retention times of the fluorescent products found, correspond to those of standard B[a]P-tetrols 1-1 and 1-2, confirming the presence of anti-BPDE-DNA adducts in human placenta DNA. **Conclusions.** Currently in addition to completing the number assays that we set out (n=100), we are initiating the analysis, that allows us to find the association between the presence of adducts in DNA, with some complications in the maternal and fetal health during pregnancy and after delivery and establish areas in which the possibility of forming such adducts is higher.

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# Role of angiotensin II in adrenergic vascular contraction during Diabetes Mellitus.

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Chronic degenerative diseases like diabetes mellitus are among the leading causes of morbidity and mortality worldwide. Diabetic metabolic abnormalities can cause damage to many organs, including blood vessels. In the vasculature, diabetes causes vascular remodeling and endothelial dysfunction. These situations are involved in disease complications that significantly reduce life expectancy. The combination of systems such as renin-angiotensin system and the adrenergic system, contribute to changes in diseases such as diabetes mellitus. In vascular system, renin-angiotensin system is a central component in physiological and pathological responses. Angiotensin II exerts its physiological actions on cardiovascular system and other organs and systems, regulating immediate effects such as vasoconstriction, the regulation of blood pressure and vascular remodeling. Renin-angiotensin system overstimulation plays an important role in the genesis of vascular dysfunction and it is involved in inflammation, endothelial dysfunction, as well as structural and mechanical changes in blood vessel walls and nervous system hypersensitivity. In the present study, we evaluated the influence of angiotensin II in vascular adrenergic response during diabetes mellitus.

The reactivity to phenylephrine was determined in aorta from control rat, diabetes rat (streptozotocin-induced 55 mg/kg , single dose) and diabetes rat treated with captopril (an ACE inhibitor; 30 mg/kg/day, 4 weeks). The results showed that, in diabetes rats, phenylephrine response decreased 44 % compared to control. While in diabetic rats treated with captopril, phenylephrine response was decreased 20%. Renin-angiotensin system inhibition during diabetes improves adrenergic contractile response by a mechanism not described.

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# Detection of galphimines through TLC, HPLC, and $^{1}$H NMR in natural populations of the Mexican plant *Galphimia glauca* Cav. (Malpighiaceae).

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*Galphimia glauca* Cav. (Malpighiaceae) is a plant used popularly in Mexico since prehispanic times to treat different illnesses, which include central nervous system disorders. *G. glauca* is distributed widely in the country; however the scientific research about the pharmacological properties of the plant have been limited to populations that are located in certain localities of Mexico. The first studies made on a natural population were carried out in Doctor Mora, Guanajuato and demonstrated that the plant has anxiolytic and sedative properties in both, mice and humans. The compounds responsible of these properties were isolated and identified and belong to a triterpenes family of *nor*-secofriedelanes named galphimines. Nowadays, it is very necessary to extend the study of the *G. glauca* included other localities of the country in order to increase the knowledge that is already known about the plant and also to determine which of them are galphimines producer. In this work, four new natural populations of *G. glauca* in the states of Hidalgo, Morelos, Querétaro and Zacatecas were studied through thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and proton nuclear magnetic resonance ($^{1}$H RMN). The TLC results demonstrated that among the new populations being studied only the populations of Hidalgo and Querétaro produces galphimines. These results were confirmed through the profiles obtained on the analysis of HPLC where the peaks belonging to the galphimines in these populations are observed. In the same way, through $^{1}$H-RMN we obtained spectra that presented specific signs corresponding to galphimines in the new population in the study carried out in Hidalgo and Querétaro. These results suggest that only these two populations will possess sedative and anxiolytic properties. The subsequent metabolomic study will allow us to go in depth in the analysis of the differences observed in the chromatographic profiles and $^{1}$H RMN spectra as well as to establish the relation of the chemical profile with the pharmacological one.

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# Pinocembrin ameliorates metabolic disturbance of diabetic nephropathy

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**Introduction.** Diabetic nephropathy (DN) is the leading cause of end stage renal disease in the world. DN increases the risk of death, to promote cardiovascular disease. Microalbuminuria is considered the earliest marker and best predictor of progression to renal failure and cardiovascular damage. The current available treatment for DN are inhibitors of enzyme converting of angiotensin II and angiotensin II receptor blockers, however they are not accurate besides they are only effective in early stages. Thus, it is necessary to find new alternatives for treating and preventing DN. Flavonoids are the major functional components of many herbal preparations for medical use. Pinocembrin has been extracted from *genera Piperaceae* and recently, from propolis. Pinocembrin shows a broad range of biological properties such as anti-inflammatory, antimicrobial, anticancer and antioxidant.

**Aim**. Evaluate the effect of pinocembrin in diabetic nephropathy in experimental type 1 diabetes.

**Methods**. Two different treatments were tested: preventive and corrective treatments. In both schemes, four groups of male Wistar rats were formed (control, pinocembrin, hyperglycemic and hyperglycemic+pinocembrin). Before to start the experiment, the rats were injected with streptozotocin (60 mg/Kg, i.p.) to develop hyperglycemia. In preventive treatment, pinocembrin was administered orally (10 mg/Kg) by 40 days once hyperglycemia was established. Whereas, in corrective treatment, pinocembrin was administered for 20 days, once DN was established (40 days after hyperglycemia was recognized). Glucose and body weight were monitored weekly. Renal profile: serum creatinine (sCr), blood urea nitrogen (BUN), creatinine clearance (ClCr), proteinuria, urinary volume; lipid profile: total cholesterol, triglycerides, very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL); hepatic profile: aspartate transferase (AST), alanine transferase (ALT) and alkaline phosphatase (ALP), malondialdehyde and histopathological changes were measured.

**Results**. In both treatments, hyperglycemic rats showed significant increase of glucose, body weight loss, BUN, urinary volume, proteinuria, cholesterol, triglycerides, VLDL, LDL, ALP, AST, ALT, MDA and hypertrophy respect to the control group. In preventive treatment, the rats administered with pinocembrin significantly reduced LDL, cholesterol, proteinuria, urinary volume, MDA and hypertrophy compared to hyperglycemic group. Whereas in the corrective treatment, administration of pinocembrin only reduced statically significantly cholesterol and hypertrophy of glomeruli in comparison to hyperglycemic group.

**Conclusion**. Pinocembrin delays the onset of some signs of metabolic disturbances before the renal damage is established and also delays the structural lesions occurred once DN is established. Thus, the present data suggest that pinocembrin can be used as an adjuvant agent in current patients with this disease.

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# Antioxidant and antimicrobial activity of *Costus pulverulentus* extracts: a plant used in Huasteca Potosina traditional medicine

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*Costus pulverulentus* (Costaceae), native from Mexico extending into Central and South America, is commonly known as caña de puerco or caña de venado. The leaves are large and spirally arranged on stems which differentiate it from its nearest relative, the zingiber or true ginger. The plant is used in Mexican folk medicine in urinary affections and for expelling urinary stones. The objective of this study was to evaluate the antioxidant and antimicrobial activity of hexane, methanol and ethanol/water extracts from *Costus pulverulentus* (CP) stem. Fresh stems were collected from Aquimon, S.L.P., Mexico and identified by a specialist. They were dried under shade and mechanically reduced to powder. The powder (80 g) was successively extracted by percolation with the solvents in increasing polarity starting with hexane, methanol and ethanol/water (70:30). The extracts where then concentrated by using a rotary evaporator at 40°C under reduced pressure. The dried extracts were suspended in distilled water and stored at 4°C until use. The total phenolic content of the extracts was determined by the Folin-Ciocalteu Method and the results were expressed as gallic acid equivalent (EAG) per liter. The antioxidant activity of the extracts were determined by the DPPH assay. Green tea was used as a positive control. Antimicrobial activity was tested in Gram-negative bacteria obtained from patients with urinary tract infections. Activity was determined by the agar dilution method. Sulfamethoxazole/trimethoprim was used as a positive control. The total phenolic content of the hexane, methanol and ethanol/water stem extracts was 199.13, 358.78 and 335.31 EAG mg / L, respectively. The methanol and ethanol/water stem extracts of CP had strong antioxidant activity against all the free radicals investigated. DPPH scavenging activity was 62.54 and 72.94%, respectively, while that of the hexane extract, was 18.36%. The high phenolic content is correlated with the antioxidant activity of the extracts. The ethanol/water extract had the greatest activity against *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* with values of 70, 50 and 63% of bacterial growth inhibition. The antimicrobial activity may be attributed to the high content of phenolic compounds.

Our results suggest that *Costus pulverulentus* is a potential source of antioxidant and antimicrobial agents. Further phytochemical analysis is required to isolate the elements of the plant that show the pharmacological activity.

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# Neurotoxic effects of early exposure to the herbicide atrazine during the development of zebrafish (*Danio rerio*)

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Atrazine (ATR) is one of the most widely used herbicides in the agricultural industry and a potential contaminant of surface water. Several studies in rodents have reported toxic effects of ATR on the immune, reproductive and nervous systems. It suggests the toxic potential of ATR for both human health and biodiversity. Due to the limitations of murine models most studies have focused on evaluating ATR toxicity in juvenile and adult stages. While few studies have characterized the toxicity of the exposure to ATR at environmentally relevant doses on the development of the nervous system. This study aims to evaluate the toxicity of ATR in the neurodevelopment of zebrafish, an emerging model favorable for toxicology assays due to its translucent embryonic development and its high fertility rate, which favors to obtain a large number of individuals. We built a low-cost fish tanks system with closed recirculation and chemical-biological filtration, which allows sustained production of embryos under optimal conditions. In order to determine ATR toxicity, fertilized embryos were exposed after the gastrulation phase and during organogenesis [1.25 - 96 hours post- fertilization (-hpf)] to 3, 30, 300 and 3,000 µg ATR/L. Additionally, groups of embryos were exposed to the same ATR concentrations from 4 hpf to 90 days post- fertilization (dpf) in custom made fish tanks in order to evaluate the effects of chronic ATR exposure on learning and memory tasks. Results show that early exposure to ATR has no acute effects in embryotoxicity endpoints at any of the doses used. On the other hand, larvae treated with 300 and 3000 µg ATR/L had increased cell death markers at 48 hpf using acridine orange whole mount and increased motor larval activity in comparison to controls at 7 dpf. These data contribute to understanding the neurotoxic role of ATR on the development of the nervous system.
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# Hepatoprotective effect of *Callistemon citrinus* on paracetamol induced liver toxicity in rats

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*Callistemon citrinus* presented high levels of terpenes, phenolic and flavonoids compounds. The extracts of flowers and leaves were toxicological evaluated and showed nulled acute and subacute oral toxicity in Wistar rats at 1000 mg/Kg dose [a].

The present study was carried out to evaluate the hepatoprotective effect of the extracts of *C. citrinus* against paracetamol induced liver toxicity in rats. Group 1 receive water, group 2 received a dose of 400 mg/kg of paracetamol, groups 2 and 4 received extracts of *C. citrinus* flowers an leaves at a dose of 300 mg/kg plus paracetamol 400 mg/kg and group 5 received a dose of sylmarin 2000 mg/kg plus paracetamol 400 mg/kg, were administered for 7 days to rats following the protocol proposed by Kanchana *et al.,* (2011)[b]. The study of the hepatoprotective activity revealed that level of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) were increased and the level of total protein and albumin showed significant reduction in the serum of rats treated with paracetamol. The histopathology of liver sections of these rats present severe damage, instead the effect of the flower and leaves extracts in restoring the normal functional ability of the hepatocytes were equal as the silymarin one drug used in the treatment of liver diseases.

a) López-Mejia A. 2015. *Estudio farmacológico de los extractos de hoja y flor de Callistemon citrinus*. Tesis de Licenciatura. Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo.

b) Kanchana N. and M. Saidq. 2011. *Hepatoprotective effect of Plumbago zeylanica on paracetamol induced liver toxicity in rats*. International Journal of Pharmacy and Pharmaceutical Sciences. Vol 3, Issue 1. 151-54.

# Peptide toxins modulate progesterone-induced Ca2+ influx through CatSper ion channel in human sperm

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Changes in the concentration of intracellular Ca2+ ([Ca2+]i) regulate sperm motility and the acrosome reaction, processes necessary for sperm to fertilize the oocyte. Activation by progesterone of CatSper, a unique and exclusive sperm Ca2+ channel, causes a transitory [Ca2+]i elevation crucial for sperm motility and fertilization. Given their remarkable potency and specificity targeting ion channels, in this project more than 900 peptide toxins have been isolated from venoms of different animal species such as spiders, scorpions and snakes. This report focuses on approximately 300 peptide toxins from different species of coral and cobra snakes. Some of the most potent inhibitory toxins of the progesterone-induced Ca+2 influx through CatSper were from cobra snake venoms. Human spermatozoa were loaded with the fluorescent calcium indicator Fluo-4 AM. The screening was performed using a high-throughput methodology with a multi-modal plate reader capable of recording from 96-well plates (Flex Station 3®, Molecular Devices, USA). From 201 peptide toxins isolated and screened from 18 species of cobras, 20 toxins showed a significantly inhibitory action of the progesterone-induced [Ca2+]i elevation through CatSper channel. Of those, one toxin from *Naja mossambica,* showed a clear concentration-dependent inhibitory effect. These results demonstrate the potential of venom-based peptide toxins as possible candidates to constitute the base for pharmacological agents to be seriously considered in the search for effective human male contraceptives.

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# Melatonin effect on lead biodistribution

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Melatonin is a hormone produced by pineal gland, regulation of the seasonal reproduction and circadian rhythms are well known functions. Among its antioxidative, antinflammatory actions, melatonin can reduce lead toxicity in vivo and in vitro. We studied the melatonin's effects on lead-biodistribution. Rats were intraperitoneally injected with lead acetate (10, 15 or 20 mg/kg/day) with or without melatonin (10mg/kg/day) daily for 15 days. In rats intoxicated with the highest lead dose, those treated with melatonin had lower lead levels in blood and higher levels in urine and feces than those treated with lead alone, suggesting that melatonin increase lead excretion. To explore the mechanism, we analyze the level of some metal transporters proteins in liver and kidney. Liver and kidney mRNA levels of metallothioneins (MT) were quantified. Melatonin co-treatment increase the MT2 and MT1mRNA expression in the liver of rats received the highest doses of lead. The mRNA levels of all analyzed genes were lower in the kidney compared to liver.

**Table 1**. Levels of lead in different samples.

| | BLOOD | | BONE | | BRAIN | | DREGS | | KIDNEY | | LIVER | | URINE | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | µg/dl | SE | µg/g | SE | µg/g | SE | µg/g | SE | µg/g | SE | µg/g | SE | µg/dl | SE |
| Control | 2.11 | 0.08 | 0.16 | 0.06 | 0.01 | 0.00 | 0.71 | 0.16 | 0.13 | 0.03 | 0.07 | 0.02 | 0.88 | 0.44 |
| Pb10 | 26.61* | 5.11 | 74.49***** | 23.66 | 0.57***** | 3.01 | 6.97***** | 0.99 | 46.18* | 3.41 | 23.44***** | 11.11 | 11.84***** | 4.07 |
| Pb10M | 35.54* | 5.48 | 94.98***** | 4.72 | 0.54***** | 0.05 | 63.48* | 0.87 | 67.36* | 12.64 | 17.18***** | 2.32 | 16.93***** | 3.65 |
| Pb15 | 35.30* | 1.77 | 128.41* | 4.61 | 0.93* | 0.13 | 46.38* | 1.36 | 62.93* | 18.53 | 18.94***** | 3.89 | 14.39***** | 3.07 |
| Pb15M | 26.83‡ | 6.27 | 70.06**‡*** | 21.17 | 0.50**‡*** | 0.13 | 40.05* | 12.67 | 51.81* | 13.17 | 23.74**‡*** | 14.79 | 32.22**‡** | 2.67 |
| Pb20 | 53.64* | 10.88 | **130.08*** | 29.83 | 0.82* | 0.24 | 65.12* | 4.75 | 75.34* | 1.35 | 42.60***** | 19.70 | 18.61* | 17.28 |
| Pb20M | 22.54‡ | 5.01 | **49.42‡** | 19.51 | 0.61**‡*** | 0.19 | 47.18**‡*** | 9.28 | 45.58**‡*** | 6.10 | **117.12*‡** | 6.28 | 46.09‡ | 1.67 |

*****: Significant difference related to control group, tested by one-way ANOVA, followed by Turkey test. P<0.02.

**‡**: Significant difference between two groups: lead *vs* lead plus Melatonin, tested by one-way ANOVA, followed by Tukey's multiple comparison test. P<0.02.

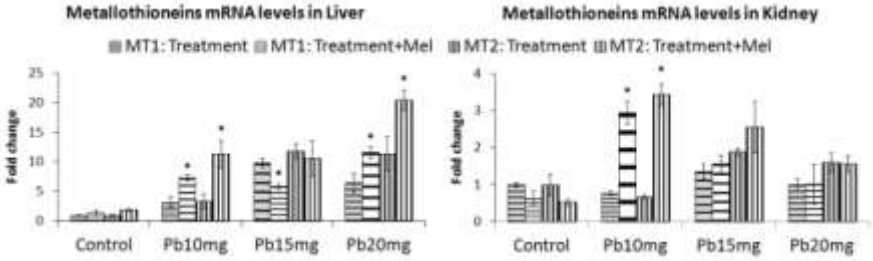


**Figure 2. MT1 and MT2 mRNA levels in animals treated with lead and melatonin in subacute administration**. *****: Significant difference related to the control group, tested by one-way ANOVA, followed by a Tukey's multiple comparison test, *p*<0.02.

# Antitumor activity of extract of Ficus crocata in breast cancer cells MDA-MB-231

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Abstract

Breast cancer is the first in deaths from malignant neoplasms in Mexican women; treatments are very aggressive and invasive, damaging not only malignant cells but also healthy cells, predisposing the patient to side effects that may even lead to death. In recent years, greater importance has taken the use of natural products as alternative or complementary treatment for different diseases therapy. Some studies have reported that extracts of *Ficus* species have antitumor activity by their pharmacological and phytochemical properties. In Mexico there are no reports of biological effect of *Ficus* species, however there are several species of this genus in our country. **Objective:** To evaluate the antitumor activity of extracts of leaves of *Ficus crocata* of Guerrero state in cell lines of breast cancer. **Methodology:** the extraction of metabolites of *Ficus crocata* leaves was performed using hexane, dichloromethane and acetone as solvents. The MDA-MB-231 cells were exposed to various doses and times to evaluate its effect on cell proliferation by tests with crystal violet 0.1% and cell migration using wound healing assays. **Results:** A significant decrease in cell proliferation after treatment with extracts was observed, compared to untreated cells; a similar effect was observed with the three phases of the extract. Furthermore a significant decrease in cell migration treated with dichloromethane and acetone extracts was observed, while the dichloromethane extract stimulated the cell migration. **Conclusions:** Althoug more studies evaluating the mechanisms of action of metabolites extracted from *Ficus crocata* are required, the extracts of *Ficus crocata* could be used as complementary therapy for the control of proliferation and migration of breast cancer cells.

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# Perinatal administration of bisphenol A alters the expression of tight junction proteins in the uterus and reduces the implantation rate

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Bisphenol A (BPA) is an endocrine disruptor which possesses estrogenic like activities in hormone sensitive and dependent tissues. Correlations between BPA and infertility have been reported, however the molecular mechanism that it affects has not been stablished. The aim of the present work was to study the effect of BPA administration, during the perinatal period, over the fertility of the F1 generation and the expression of tight junction (TJ) proteins during early pregnancy. Three groups of pregnant Wistar dams (F0) were used, which received: BPA-L (0.05 mg/kg/day), BPA-H (20 mg/kg/day) or vehicle, from pregnancy day (PD) 6 to lactation day 21, in their drinking water. Beginning on the day of weaning, F1 female pups were supplied with unadulterated drinking water and left unaltered until 3 months of age; at that time they were mated with an expert male with proven fertility. F1 pregnant rats were sacrificed at PD 1, 3, 6, and 7. Serum hormonal levels, ovulation and implantation rates, and the expression of proteins of the TJ (claudin-1, -3, -4, -7, and ZO-1) were evaluated. We found that perinatal treatment with BPA-H decreased the level of progesterone (P4) in serum at PD 1, the implantation rate at PD 6 and 7, increased the expression of claudin-1 at PD 6 and 7 and eliminated the peaks of claudins-3 and -4 expression at PD 3 and 6, respectively. Meanwhile, BPA-L treatment decreased estradiol (E2) and P4 levels in serum at PD 3 and 6, respectively, increased claudin-4 expression at PD 1 and 3, decreased claudin-7 expression at PD 1 to 6, and diminished the expression of claudin-4 in stromal cells at implantation sites at PD 7. In conclusion, BPA treatment during the perinatal period decreased the number of implantation sites, when the animals reached adulthood and became pregnant, because it blocked the particular and synchronized expression of TJ proteins in the uterine epithelium.

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# Differential expression of drug transporters associated with chemoresistance in soft tissue sarcomas from child: Comparison between tumor and normal tissue.

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**INTRODUCTION**: The soft tissue sarcomas (STS) are a heterogeneous group of solid tumors of mesenchymal origin representing between 8% and 10% of pediatric cancers, of which there are more than 50 different histological subtypes that vary in their clinical presentation . Although chemotherapy improves survival and quality of life of patients with STB, eventually most develop progressive disease, metastasis and chemoresistance. Among the mechanisms that exist of chemoresistance stands overexpression of protein transporters in tumor cells of ABC type (MDR1, MRP1 and MRP2), which have the property of expelling drugs of different chemical structure, including antineoplastics used in STS therapy. Although it has been shown that the ABC type binding proteins are markers of poor prognosis in some childhood cancers like neuroblastomas, Wilms tumor, and Ewing's sarcoma, so far little is known about modulation of transporters at the tumor site *vs* adjacent normal tissue of patients with STS. **OBJECTIVE**: Determine the intratumoral profile of expression and the adjacent normal tissue of MDR1, MRP1 and MRP2 genes in patients with STS in pediatric age. **MATERIAL AND METHODS**: We evaluated 17 patients diagnosed with STS type not rhabdomyosarcoma, without prior chemotherapy, biopsies were obtained both the tumor site as normal adjacent tissue. In these samples gene expression MDR1, MRP1 and MRP2 was analyzed by RT-qPCR using as endogenous control ß-actin. **RESULTS**: The gene expression of transporter proteins was detected in all tissues, showing important differences in transcript levels between the tumor tissue *vs* normal tissue. Intraindividual expression analysis showed increased levels of MDR1 in 64.7% (11/17), 76.4% MRP1 in (13/17) and MRP2 in 70.5% (12/17), while overexpression of MDR1, MRP1 and MRP2 was identified in 6, 4 and 5 respectively tumor tissues. Interindividual analysis revealed the MDR1 expression as the transporter with largest increase in expression levels (1.75 times), followed by 1.05 times with MRP1 and MRP2 with 0.94 times. **CONCLUSION:** Overexpression of MDR1, MRP1 and MRP2 genes in tumor tissue of STS, could be decisive in response to chemotherapy, as well as being the main cause of treatment failure, as antineoplastic as vinca alkaloids and anthracycline frequently used in the treatment of STS are substrates of these transporters, MDR1 mainly, who is responsible for decrease intracellular accumulation of drugs. As establishing gene manipulation strategies to inhibit the activity of ABC transporters and induce greater cytotoxicity of anticancer necessary.

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# Antinflamatory activity of isolated sesquiterpenic compounds from P. *decompositum*

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Inflammation is a physiological response to several stimuli, its aim is the disposal of harmful agents and to restore physiological functions of the compromised tissue or organ. However, the balance might be affected or lost permanently, which often has pathological consequences, which is why there is a large number of drugs used as treatment, such as SAs and NSAs. These treatments often have adverse effects regardless of its therapeutical purpose, this brings the research for new anti-inflammatory compounds.
Mexico has a great variety of vegetal species with diverse medical usage, *Psacalium decompositum* being one of the species used for antidiabetical purposes that has shown anti-inflammatory effect. The effects of the specie and its sesquiterpenic compounds over classic inflammation mediators remains unknown.
The aim of this research was to evaluate *in vivo* and *in vitro* anti-inflammatory activity of cacalol acetate, cacalol and a fructo-oligosaccharides fraction isolated from *Psacalium decompositum* roots. For *in vivo* model, CD-1 male mice were used, inflammation was induced by topical ear administration of TPA. For an in vitro study, RAW 264.7 macrophages were stimulated with LPS to induce inflammatory response, subsequently they were treated with different compounds at different timing, for RNA expression screening of TNFα, IL-6, IL-1β and IL-10.
Both cacalol and cacalol acetate inhibited significantly the development of auricular edema up to 40% compared to control, unlike fructo-oligosaccharides fraction, which showed 28%. Furthermore, the cultures treated with the compounds significantly decreased the expression of TNF-α, IL-6 and IL-1β but didn´t modify the expression or concentration of IL-10. These results suggest cacalol, cacalol acetate and fructo-oligosaccharides reduce inflammation through the modulation of inflammatory cytokines, suppressing pro-inflammatory ones and not modifying anti-inflammatory cytokines. It remains necessary the study of these compounds on transcription factors involved in the production of these cytokines.

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# Bioguided isolation of antifungical compounds form *Zizyphus obtusifolia*.

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The increase in patients with acquired immunodeficiency syndrome (AIDS), antibiotics and agents that decrease the activity of the immune system, are factors related to the increase of opportunistic mycosis in recent years, causing a health problem to nosocomial level. Several studies have shown the *in vitro* antiproliferative properties of medicinal plants. In Sonora, the native groups (Yaquis, Mayos, Seris, Guarijíos, Pimas and Kikapúes), have extensive knowledge of medicinal plants. In the present study the antifungal activity of *Zizyphus obtusifolia* against *Aspergillus niger* and *Aspergillus flavus* was evaluated. Phytochemical scavenging was conducted to see the main group of metabolites present in the extract. For activity assays dilution method was used in measuring the radial agar every 24 hours for 7 days growth. For bioguided isolation, the extract was fractionated by solid-liquid partition and fraction greater activity was separated by column chromatography with silica gel. The results show a greater inhibitory effect against *A. niger*, the phytochemical analysis of the most active fraction suggests that the compounds responsible for the activity are triterpenoid saponins.

Keywords: Aspergillus, antifungical, Medicinal plants

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# Evaluation of antioxidant activity of Callistemon *citrinus* on subacute oral toxicity in male Wistar rats.

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*Callistemon citrinus* has been use in other countries due to its curative effects for antidiabetic, anti-inflammatory (Kumar et al., 2011), cardioprotective, antifungal and antioxidant (Cid-Estrada, 2014), however in Mexico has an ornamental used only. The present study was focused on investigating the role of the extracts of *C. citrinus* of leaves and flower, *in vitro* antioxidant activity superoxide and nitrite radicals, and the effect of the extracts of leaves and flower in Wistar rats. The superoxide anion scavenging activities of the ethanolic extracts of *C. citrinus* of leaves and flower displayed moderate antioxidant activities. However, nitrite anion scavenging activities of the ethanolic extracts of *C. citrinus* of leaves and flower displayed stronger antioxidant activities. This antioxidant activity test were compared to standard antioxidant such ascorbic and gallic acid.

The analyzed for lipid profile in the administration with the extracts of *C. citrinus* of leaves and flowers (1000 mg/kg) for 28 days were similar to the control. The activity of superoxide dismutase (SOD) has lighter decreased in the administration with the extracts of *C. citrinus* of leaves and flowers (1000 mg/kg) for 28 days than the control. Meanwhile the catalase (CAT) and peroxidase activities were greater in the rats feed with extracts of *C. citrinus* of leaves than the control and the extracts of *C. citrinus* of flowers. The rate GSH/GSSG were decrease in the rats feed with the extracts of *C. citrinus* of leaves and flowers, finally the lipid peroxidation (LOP) were higher in the rats feed with the extracts of *C. citrinus* of flowers than in the control and the extracts of leaves.

The results in this study explain the beneficial antioxidant effect of the extract of *C. citrinus* of leaves in liver.

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Kumar, S., V. Kumar y O. Prakash. 2011. Pharmacognostic study and antiinflamatory activity of *Callistemon lanceolatus* leaf. Journal of Tropical Biomedicine. 1: 177-185.

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# Biotin supplementation has no detrimental effects on oxidative stress markers but induces genotoxicity in mice.

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**Introduction.** Biotin is a water-soluble vitamin that acts as a covalent bound coenzyme of carboxylases. Unrelated to this role, biotin at pharmacological concentrations, about 30-650 times greater than its requirement of 30 micrograms per day, modifies gene expression. Several studies in humans and animal models have found that pharmacological concentrations of biotin have favorable effects on triglycerides and glucose homeostasis. These effects suggest that biotin could be used for the treatment of dyslipidemia, glucose intolerance 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. **Aim.** To analyze the effects of biotin supplementation on oxidative stress and genotoxic markers, in an animal model in which it has been found favorable effects of biotin on triglycerides and glucose homeostasis.
**Methods.** Two groups of male mice were fed from weaning to 11 weeks of age (8 weeks of treatment) with a control diet containing (1.76 mg biotin/ food kg) or with a biotin supplemented diet (97.8 mg biotin/ food kg). A third group received biotin-control diet and was treated with a single dose of carbon tetrachlocloride (CCl4, 2ml/kg body weight), and was used as a positive control. At the end of the treatment mice were food deprived for 16 h, anesthetized with Sevorane and blood, liver and bone marrow were extracted. Finally, mice were killed by cervical dislocation. The activity of superoxyde dismutase (SOD), catalase (CAT), and nitric oxide (NO), y reduced glutathione (GSH) and oxidized (GSSG), were determined as oxidative stress markers. The genotoxic effect was determined by reticulocyte micronuclei formation.
**Results.** We found that eight weeks of biotin supplementation in the diet did not modify any of the oxidative markers tested, but produced a significant increase in micronuclei formation (Control=0.6± 0.04; supplemented 0.5±0.02).

| Assay | Control | Supplemented |
|---|---|---|
| SOD (U/ml) | 0.37±0.036 | 0.38± 0.05 |
| CAT (U/mg protein) | 144± 18.08 | 151.8± 13.45 |
| GSH (nmol/mg wet tissue) | 15.06±3.6 | 151.8± 13.45 |
| GSSG (nmol/mg wet tissue | 1.25±0.57 | 1.23±2.12 |
| NO (uM/mg wet tissue) | 24.51±2.07 | 25.61±1.44 |

**Conclusion.** There is not a detrimental effect of pharmacological effets of biotin on oxidative stress markers, however the increase of micronuclei raises concern on biotin genotoxicity. Additional studies will be required before to propose biotin as a therapeutic agent.

**Financiamiento** CONACyT 219787

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# Evaluation of the Antimicrobial Activity of Short Variants of Arachnid Antimicrobial Peptides.

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The emergence of drug resistant bacterial strains, as a consequence of the unmeasured use of commercial antibiotics, has redirected the pharmacological research towards the development of new antimicrobial agents. Natural and synthetic antimicrobial peptides (AMPs) offer a promising solution to this drawback because their mechanisms of action are not limited to a single receptor. However, these molecules have some disadvantages such cost of chemical synthesis, peptide stability and cytotoxicity to eukaryotic cells when compared to conventional antibiotics. Different physicochemical and structural properties of the peptides, such as the net charge, hydrophobicity, helical structure content, amphipathic character, among other have been proposed to improve their antimicrobial potential. In this work eight short peptides (14-16 amino acids) were designed *in silico* using as template the sequence of two previously characterized arachnid antimicrobial peptides and taking in count the physicochemical and structural properties mentioned above. Four peptides were designed based in the peptide Css54 (25 mer) from the scorpion *Centruroides suffusus suffusus*, and four based in the peptide La47 (20 mer) from *Lachesana* sp. spider, the eight peptides were chemically synthetized and purified by RP-HPLC, the identity of the peptides was confirmed by mass spectrometry. The antimicrobial activity of all variants was evaluated towards the bacterial strains, *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 in liquid culture media. Among the short variant evaluated, only two short variants of Css54 and two short variants of La47, showed antimicrobial effects, similar to the parental peptides, over both bacterial strains at concentrations between 6.25-50 µM. Thus data suggest that is possible reduce the number of amino acids of natural antimicrobial peptides keeping the antimicrobial activity of the template sequence.

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# Cytotoxic activity by a peptide from the venom of the centipede *scolopendra polymorpha* in the HeLa cell line

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Venom is a biological weapon that is used by many species of animals to defend themselves or capture their prey. In the group of arthropods, several organisms like spiders, scorpions, bees, wasps and centipedes employ this biochemical substance, composed of many enzymatic proteins, non-enzymatic proteins and other non-peptidic components. Some compounds found in venoms, possess great potential as anticancer agents. Cancer is a term broadly employed for a large group of diseases, which can affect either single cells or whole tissues with a common feature: abnormal growth. We know that some peptides available in venoms exhibit a high affinity to cancerous cell lines *in vitro* with a cytotoxic activity, this due apparently for the affinity with some phospholipids, like phosphatidylserine, negatively charged and located towards the external face of the cancerous cell membrane. Therefore, venom analysis and purification is important to develop new drugs that are more selective towards cancerous cells than those already available on the market while causing fewer side effects. In the present study the venom from the centipede *Scolopendra polymorpha* will be analyzed by flow cytometry using Anexin V-FITC, IP and JC-1 assays with the HeLa cell line, to determine the percentage of apoptosis, necrosis and the mitochondrial permeability. Previous experimentation and data in our laboratory revealed that the venom induces the activation of caspases 3 and 7 in HeLa cells, 24 hours after treatment with whole venom, showing a significant increase ($P<0.05$) in activation, mainly at concentrations of 50 and 100 µg/ml, indicating death by apoptosis. Additionally, the venom will be purified by HPLC using a cationic column and each fraction will be tested in a MTT assay to measure the cytotoxic activity and determinate which fraction(s) produces the best inhibition in the cellular proliferation.

# *Cucurbita Ficifolia* Bouché effects over energetic balance and inflammatory mediators on co-cultured adipocytes and macrophages.

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Obesity is a key factor to develop pathologies like Diabetes Mellitus type 2, the most common endocrinologic disorder in Mexico. It is the result of excessive amount of energy stored in adipose tissue and its associated with insulin resistance as it is with adipose tissue inflammation. Obese adipose tissue is characterized by enhanced macrophage infiltration, which causes a loop between chemoattractant factors, such as MCP-1, and cytokines like TNF-α between adipocytes and macrophages. This increases inflammatory changes and the further production of cytokines and heightened lipolysis, perpetuating a vicious cycle that augments insulin resistance and sets a chronical state of inflammation.

In previous studies, *Cucurbita ficifolia* has shown to lower glycemic levels in healthy and diabetic mice and patients. It has also shown antioxidant effect in 3T3-L1 adipocytes, however, it is important to study the effects *Cucurbita ficifolia* exerts on a co-culturing model that include adipocytes and macrophages imitating obese adipose tissue.

The aim of this inquiry was to study the effects caused under the administration of an aqueous extract of *Cucurbita ficifolia* over the expression of GLUT-4, FATP-1, and PPARγ on 3T3-L1 adipocytes and the production of TNF-α and IL-6 on macrophages, both cellular lines under co-culture conditions.

The adipocytes treated with the aqueous extract showed an increase on the expression of PPARγ, GLUT-4 and FATP-1, while in macrophages IL-6 decreased. It is possible that the effect shown by *Cucurbita ficifolia* is due to the increased production of metabolic enzymes that allow adipocyte clearance of glucose and free fatty acids from the blood stream, given by the activation or increased expression of their transcription factors, such as PPARγ, allowing also for a better insulin sensitivity and response, improving the inflammatory profile by modulating the production of cytokines produced by infiltrated macrophages.

*Keywords: Obesity, inflammation, Cucurbita ficifolia.*

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# Preclinical screening of CD44 antagonist in breast cancer cells

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Breast cancer (BC) is the most important cause of cancer mortality in women around the world. BC has a high rate of relapse and treatment failure, indicating that current therapies are insufficient to treat all patients and new therapies are required. Breast cancer stem cells (BCSC) constitute a subpopulation of cancer cells from the breast tumors. BSCS show increased tumorigenicity, can self-renew, and produce other cell linages that conform tumors. BCSC promote: i) more aggressive tumoral stages; ii) chemo- and radio-resistance; and iii) metastasis formation. Therefore, new therapeutic strategies should consider the participation of BCSC in the onset, maintenance and outcome of the BC. CD44, a cell membrane receptor for hyaluronic acid (HA), is overexpressed in BCSC in comparison to other cancer and normal cells. In the present work we aimed to select and evaluate the biological activity of potential CD44 antagonists.

Candidate compounds were selected by structure-based virtual screening (SBVS) using the ligand-binding domain of CD44 and a database of FDA-approved drugs. We obtained 8 potential antagonists to be evaluated *in vitro.* Using the MDA-MB-231 cell line as biological model, we analyzed the effect of drugs on CD44-HA interaction, cytotoxicity, cell adhesion, and mammosphere formation. MDA-MB-231 cells proceed from a triple negative BC tumor, which is the BC subtype with worst prognosis, and overexpressed CD44. By MTS assays, we evaluated the cytotoxicity (48 h) of the selected drugs. We identified 2 drugs (9805 and 5290) with $IC_{50}$ <5μM; 4 drugs (34, 10111, 11546 and 1632) with 40μM< $IC_{50}$ <60μM; and 2 drugs (1276 and 4867) with $IC_{50}$ >128μM. The capability of the drugs to block CD44-HA interaction was evaluated in cell-free solid phase assays. We discovered that 2 of the selected drugs (4867 and 11546) antagonize CD44 ligand binding. The evaluation of cell adhesion to HA-coated plates showed that the same drugs that inhibit the physicochemical interaction reduce the cell adhesion to HA without affecting cell viability, corroborating their blocking capability. One more drug (34) also inhibited cell adhesion but only at concentrations that induce cell death. Finally, in mammosphere assays, we found that 5 of the studied drugs reduced the clonogenic capability of breast cancer cells, suggesting that the drugs have a direct effect on the BCSC population. However, 3 of these drugs are ineffective blocking the CD44-HA interaction and therefore their effect seems to be independent of CD44.

Our results showed that only 2 drugs (4867 and 11546) block CD44-HA binding and cell adhesion, demonstrating that they act as CD44 antagonists. Those drugs reduced the mammosphere formation. Further experiment will focus on demonstrate that the clonogenic reduction induced by these drugs are mediated by alterations in CD44-triggered signaling pathways. In the case of the other 3 drugs that do not antagonize CD44 but reduce mammosphere formation, we will explore the mechanisms by which they affect BCSC.

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# Cadmium exposure produces insulin resistance, impaired insulin signaling and steatosis in liver of Wistar rats

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**Introduction:** Exposure of cadmium (Cd) has been associated with an important number of toxic effects in animals and humans. Recently, Cd has been linked with insulin resistance and dyslipidemia. However, it is unknown if Cd interfere with the insulin signaling pathway in liver, and if it is related with the hepatic lipogenesis. **Objective**: Investigate the changes in liver insulin signaling pathway and his relationship with the lipogenesis during a Cd oral exposition. **Methodology:** Wistar rats were exposed to $CdCl_2$ (15 ppm and 32.5 ppm) in drinking water for 15 and 30 days. Just before of sacrifice, the oral glucose tolerance and insulin response were evaluated at 0, 30, 60 and 90 min. Likewise, insulin resistance indexes HOMA-IR and LIRI were determinate. In fasting serum, glucose, triglycerides (TG) and Apolipoprotein B (ApoB) and Free Fatty Acid (FFA) were measured. After a perfusion with SSI, hepatic tissue was obtained and, the levels of TG and FFA were quantified. Meanwhile, in liver slices were evaluated: TG deposition by Red Oil staining, as well as, Insulin Receptor (pY1361), Insulin Receptor (pT1375), Akt (p-S473), SREBP1-c and ERK 1/2 (pThr 202/pTyr 204) by means of immunoreactivity. **Results:** The results showed that Cd exposure (dependent of time and concentration) modified the glucose tolerance, and produced hepatic insulin resistance, which was revealed by HOMA-IR and LIRI indexes. This was also confirmed by the higher TG, ApoB and FFA concentrations in serum, which were in concordance with the increase of TG and FFA in liver. On the other hand, increments in immunoreactivity to insulin receptor (pY1361), inulin recptor (pT1375), ERK 1/2 and SREBP1c were observed, however, the immunoreactivity to Akt (pS473) was diminished. **Conclusion:** Oral cadmium exposure produces hepatic insulin resistance, dyslipidemia, hepatic steatosis and important changes in the insulin signaling pathway, which are dependent of the time and metal concentration.

# Two antidepressants with different mechanisms reduce behavioral deficits in a rat model of major depression

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**Abstract**

Major depressive disorder (MDD) is a commonly occurring and life-threatening disease, characterized by a pervasive and persistent low mood. The theory that a deficiency of serotonin (5-HT) in the brain is key in the onset of MDD. Low 5-HT levels can induce structural and functional changes in the hippocampus, amygdala and prefrontal cortex (PFC), areas involved in behavioral changes seen in depression. Moreover, selective 5-HT reuptake inhibitors (SSRIs) remain the first choice in antidepressant drug treatment. Agomelatine is a novel antidepressant drug that act as agonist to melatonin receptors and antagonist to 5-HT2C receptors. This pharmaceutical also represents the first antidepressant shifting away from the traditional monoamine hypothesis for depression pharmacology. Numerous studies have showed that olfactory bulbectomy (OBX) in the rat results in a number of physiological and behavioral changes comparable to human depression. The aim of this study is to detect the behavioral effects of agomelatine and fluoxetine a model of major depression (OBX) after different periods of administration. Male Wistar rats weighing 250-300g were divided into five groups as follows: Sham operated, OBX, OBX-Vehicle (1% carboxymethylcellulose), OBX-Agomelatine and OBX-Fluoxetine. Three weeks after the bilateral OBX,the behavioral tests were performed [avoidance to light test and analysis of motor behavior in the open field test(OFT)]. Antidepressants were administered by 4 periods 0,7,14 and 21 days. All animals were sacrificed at the end of behavioral procedure and craniotomy was carry out to include in the analysis only animals with bilateral OBX without injury in other areas of the brain. Bilateral OBX surgeries in the rat have been successful and OBX rats have decreased weight compared to sham surgery control without extraction groups. Both antidepressants Fluoxetine and Agomelatine reduced the hyperactivity in the OFT after 7,14 and 21 days of treatment, altered the response to adverse stimuli and decreased grooming can be equated a symptom of depression in humans. Altoguether these results suggest that the pharmaceutical Agomelatine may be useful in the treatment of MDD.

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# VENOM FRACTIONS OF *Scolopendra polymorpha* WITH MYOTOXIC ACTIVITY ON MICE

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**Introduction.** *Scolopendra polymorpha* is a venomous arthropod widely distributed in Mexico and the United States. Several centipede venoms have been found to induce nociception, myotoxicity and edema, among other activities. Our group has observed that intramuscular injection of *S. polymorpha* whole venom (WV) results in muscular and nervous tissue damage in mouse, thus the prime objective of this investigation is to identify and characterize the toxic component which produces histological alterations over skeletal muscle in mice.
**Methods.** WV was obtained by mechanical stimulation of the centipede forcipules and collected by capillarity. Then it was lyophilized, quantified and submitted to reverse-phase high performance liquid chromatography (RP-HPLC). Myotoxic effect was evaluated *in vitro*. Briefly, the e*xtensor digitorum longus* (EDL) muscle was incubated in a saline solution (SS) during 45 min and washed 3 times at 15 min intervals prior incubation with either whole WV or some of the HPLC fractions, using SS as a negative control. Later on, muscle was processed with liquid-nitrogen frozen isopentane and cut in a cryostat. 7 µm thick sections were cut and stained with hematoxylin-eosin (H-E). Complimentary, creatin kinase (CK) activity was determined in aliquots taken during the incubation procedure, one of them before the addition of WV, fractions and/or SS (time=0 min) and two more at 15 and 45 min after.
**Results.** Twelve fractions were obtained by RP-HPLC. Fractions F4, F6, F7 and F8 were tested on skeletal muscle. H-E staining demonstrated loss of fascicle architecture, along with signs of necrosis and inflammation in muscle exposed to WV and its fractions, whereas SS exposed samples showed a higher level of conservation. Muscle incubated with WV and fractions F4, F6 and F7 increased CK release which is an indicator of muscle damage, while SS and F8 did not cause such boost at all.
**Conclusions.** We detected signs of histological changes after the incubation of EDL muscle with both WV and HPLC fractions. Exposition to fractions F4, F6 and F7 resulted in elevated CK levels *in vitro*. However, it appears to be more than a single component responsible for myotoxicity in *S. polymorpha* venom.
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# Implementation of an inexpensive system to evaluate the effect of environmental contaminants on motor behavior in zebrafish (*Danio rerio*)

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## Abstract

At present, a wide variety of pollutants are detected in the environment, these chemicals have implications for both health and biodiversity, however, due to their high number, it is impossible toxicological analysis of them all through traditional methods. In this study a low cost system was implemented to evaluate the neurotoxic effects of environmental pollutants, using the motor activity as neurotoxicity marker. We use the zebrafish (*Danio rerio*), a model with advantages toxicology research, for their low maintenance costs, high fertility as well as being sensitive to evaluate the effect of neurotoxicity biomarkers, particularly those affecting motor development. We built a low-cost fish tanks system with closed recirculation and chemical-biological filtration, which allows sustained production of embryos under optimal conditions. Followed by implementation of a simple video tracking system, using an adaption of open source software. Recording conditions and data analysis were optimized using hand-made larvae plates and custom toolbox developed in MATLAB in order to quantify total distance, time in margin and center as a measure of locomotor activity and tighmotaxis in zebrafish larvae. We validate video tracking system on relevant enviromental pollutants, using zebrafish embryos exposed from 2 hours post fertilization until 7 days post fertilization to inorganic arsenic (0.05 ppm, 0.5 ppm, 5 ppm), and atrazine (3 ppb, 30 ppb, 300 ppb, 3 ppm). Results show increased motor activity in larvae exposed to atrazine (300 ppb) and inorganic arsenic (0.5 ppm and 5.0 ppm) suggesting that inexpensive video tracking system was sensitive to detect significant changes in motor behavior in larvae exposed to enviromental relevant pollulants. In conclusion these results suggest the potential use of video tracking tool for quantifying motor activity in zebrafish larvae as a marker for neurotoxicity with a good benefit-cost relation which could increase the number of pollutants studied with neurotoxic potential. Project funded by the program 10167, NPTC PRODEP-SEP.

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# Protective effect of Theophylline in aorta versus toxic effect of $T_iO_2$ in wistar rats

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**Introduction:** Titanium dioxide nanoparticles ($T_iO_2$) are important commercial compounds; which have light scattering propierties, chemical stability and alleged absence of toxicity. The contact of people with these nanoparticles is becoming more common due of their many applications. The toxic effects of $T_iO_2$ nanoparticles have been evaluated in various organes.

Theophylline is a xantine drug, which it used as broncodilatador. It has been observed antioxidant and antiinflamatory propierties at lower doses than conventional.

**Objective:** To asses the possible toxic effects of $T_iO_2$ nanoparticles in thoracic aorta of male wistar rats, and the posible protective effect of Theophylline.

**Materials and Methods:** The study was conducted with 24 male rats of 250g which were divided into 4 groups (n=6): A) Control group, B) Theophylline group (2mg(kg/d for 5 days, po), C) $T_iO_2$ group (a single doses of 5mg/kg, iv) and D) Theophylline + $T_iO_2$ group (2mg/kg/d for 5 days, po; and 5mg/kg, iv; respectively). Glutathione (GSH) and malondyaldehido (MDA) levels in aorta, haematoxylin/eosin stainings, sistolic blood pressure, diastolic blood pressure and mean blood pressure were evaluated. Statistical analyses by Tuckey and ANOVA test.

**Results:** Reduced GSH values were found in C and D groups, meanwhile A and C showed normal values. MDA values showed no significant diferences between the four groups. The values of sistolic blood pressure, diastolic blood pressure and mean blood pressure were found elevated in C; in A, B and D were normal. There were histological change: a major contraction was found in C, meanwhile in A, B and C the contraction was not so intense.

**Conclusions:** $T_iO_2$ nanoparticles produce condition in the function of the thoracic aorta in male wistar rats, which is reversed with the prophylactic treatment of Theophylline.

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# Logical model of the Wnt/β-catenin pathway in neonatal and adult human CD8$^+$ T cells.

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Infants are very susceptible to infections by intracellular pathogens; CD8$^+$ T cells are responsible for eradicating pathogen-infected cells. CD8-T cells enter the blood circulation as naïve cells, incapable of immunological functions. To become cytotoxic effector cells, capable of antigen-dependent clearance of infected cells, they have to be properly activated by mature antigen presenting cells. The molecules responsible for T cell activation are the T cell receptor (TCR) and accessory molecules that deliver either costimulatory or inhibitory signals. Neonate T cells, have a poor response to activation and a lower cytotoxic response than adult cells. Despite this, under strong coestimulatory-conditions cells achieve adult-like responses. This suggests that neonatal cells have a rather high activation threshold.

The Wnt/β-catenin pathway has been reported to be antagonistic to the TCR pathway. Previous results in our laboratory have found that this pathway is enriched in open chromatin in neonatal CD8$^+$ T cells. Additionally, when the transcription TCF1 was silenced with siRNAs, neonate but not adult CD8-T cells were more responsive to TCR stimulation. This suggests that this pathway could be delivering negative signals for the activation of the neonatal cells.

To gain further understanding of the crosstalk between the wnt/β–catenin and the TCR pathways, we decided to build a logical model that will describe the wnt/β–catenin pathway in T cells. This will be later merged with a TCR model, already built in our lab, to be able to predict the important nodes that could mediate the inhibitory effect of the wnt/β–catenin pathway on T cell activation.

He we present the molecular map of the Wnt/β-catenin referenced to human CD8$^+$ T cells built on the CellDesigner software, which contains information of the components of the pathway and their post-translational modifications. We also present the advances in the building of the logic model with the GINsim software, where logical functions were assigned to the interactions between nodes (genes, proteins, mRNA, transcriptional factors, etc.). This will lead to the generation of states that allow us to analyze the behavior over time of the pathway, after definition of the initial conditions.

# "Zinc role in mouse spermatogenic cells"

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Sperm is a haploid differentiated essential cell for fertilization. When sperm fuses with the ovule, it will provide the other half of the chromosomes required to create a new organism with a unique genome. Spermatozoa generate from stem cells (spermatogonia) through a process called spermatogenesis in the seminiferous tubules inside the testis. Spermatogenesis has three important steps namely: mitosis, meiosis and spermiogenesis.

In humans, zinc ions have a crucial role during spermatogenesis. Zinc ions are involved during growth and development, chromatin condensation, modulation of oxidative stress and structural maintenance of seminiferous tubules. It has been also reported that zinc deficiency reduces testosterone production, that is a fundamental hormone for spermatogenesis. In addition, zinc deficiency also increases oxidative stress, seminiferous tubules atrophy and inhibits spermatids differentiation. On the other hand, an excess of zinc has detrimental effects on spermatogenesis, such as a decrease on sperm motility, arrest of spermatogenic cells and seminiferous tubules atrophy. Therefore, zinc homeostasis must be strictly regulated. To maintain this homeostasis, two types of zinc transporters families, the Zip family and ZnT family, are involved. The Zip family is composed of fourteen members, and they have eight transmembrane segments, two zinc binding sites, they are involved in the entrance of zinc into the cells. Meanwhile, the ZnT family include ten members, with six transmembrane segments, three zinc binding sites, and these transporters remove zinc from the cytoplasm.

Until now, there is little information about the role of zinc ions during the different stages of spermatogenesis in mouse, for that reason in this work we will study the presence of members of the families Zip and/or ZnT in spermatogenic cells, using *in-situ* hybridation and RT-PCR. In addition, we are going to explore the zinc concentrations in several stages of sperm development using the zinc sensitive dye Fluozin-1.

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# Primary Hepatocytes Undergo an Epithelial to Mesenchymal Transition in Response to TGF-beta Signaling

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Including cell proliferation, differentiation, and apoptosis. Also, TGF-□ is involved in wound healing, organ fibrosis, tumor progression and metastasis.

The TGF-□ bioactive form is one of the most important inducers of the epithelial to mesenchymal transition (EMT). The EMT is a physiological process that causes epithelial cells reprogram their gene expression and acquire mesenchymal characteristics, it plays an important role in embryonic development and wound healing, and it has been suggested that contribute in pathological conditions such as liver fibrosis and cancer metastasis.

Several studies show that primary cultures of hepatocytes undergo EMT when these cells are stimulated with TGF-β. During the transdifferentiation process, it has been noted that hepatocytes change their morphology and acquire a fibroblast-like phenotype, this change occurs along with progressive loss of epithelial markers (such as E-cadherin, cytokeratins and others) and gain expression of mesenchymal markers (such as collagen type I, vimentin, N-cadherin and others). And by lineage tracing experiments have been demonstrated that hepatocytes can be one of the sources of fibroblasts that may contribute to the development of fibrosis.

We are interested in characterizing the TGF-β signaling pathway during the transdifferentiation process of hepatocytes in primary culture. Also, we will identify target genes and proteins of TGF-β signaling pathway that could be involved in the EMT and that have been associated with liver pathologies.Our work is supported by grants from Conacyt and PAPIIT/DGAPA/UNAM.

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# Identification of microRNAs Modulated During Osteoblastic Differentiation of Human Mesenchymal Stem Cells (CD105$^-$)

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**Background:** Elucidating the molecular mechanisms that regulate the osteoblastic differentiation of human mesenchymal stem cells (hMSCs) is essential for the development of novel therapies for treatment of bone lesions and osteoporosis. The human mesenchymal stem cells have capacity for self-renewal and to differentiate into multiple cell lineages including osteoblast, adipocyte, chondrocyte, and neuron. We previously isolated a subpopulation of hMSCs from the amniotic membrane (AM-hMSCs) based on the co-expression of the surface markers CD44, CD73 and lack of CD105. The AM-hMSCs (CD105-) subpopulation was able to efficiently differentiates to osteoblastic lineaje. MicroRNAs (miRNAs) are small non-coding RNAs that may regulate the osteoblastic differentiation by mediating translational repression or mRNA degradation of their target genes.

**Results:** Here we analyzed the expression of 667 microRNAs using RT-qPCR stem loop on Tandem Low Density Arrays during the maturation (7 day) and mineralization (14 day) stages of osteoblastic differentiation of AM-hMSCs (CD105-). Our data showed that 10 and 21 miRNAs were upregulated at early and late stages of differentiation, respectively. Remarkably, this set of miRNAs potentially targets genes involved in the differentiation of hMSCs to osteoblastic lineaje, including TGF-β/Smad (SMURF1/2, BAMBI, ACVR2A/1B) and WNT (AXIN2, APC, GSK-3β) signaling pathways. In particular, we focused in the study of miR-548d-3p, a miRNA upregulated at late stage of osteogenic differentiation with unknown functions. Interestingly, bioinformatics analysis identifies that the inhibitors of osteoblastic differentiation such as SMURF1/2, BAMBI, ACVR2A/1B, AXIN2, APC and GSK-3β genes contain in their 3´UTRs potential binding sites for miR-548d-3p. Functional analysis using miR-548d-3p antagomirs and targets validation by luciferase reporter and western blot assays, in progress, will help us to define the role of miR-548d-3p in the osteoblasts differentiation of AM-hMSCs.

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# The cancer associated Gpn3-Q279* mutant is defective to support RNA polymerase II nuclear targeting.

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Gpn3 was previously identified as "pro-apoptotic protein required for cell survival" (*Parcs*), a protein able to interact with the oligomerization domain of Apaf-1. The GPN3 gene is classified as essential in *Saccharomyces cerevisiae* and other biological models. Gpn3 is the smallest member of the Gpn family, which also includes Gpn1 and Gpn2. In yeast and human cells Gpn3 silencing has been reported to result in a decrease in global transcription by preventing the nuclear localization of RNA polymerase II (RNAPII), the 12-subunit enzyme that synthesizes all mRNA in eukaryotic cells. The catalogue of somatic mutations in cancer (COSMIC) has reported a series of mutations in the GPN3 gene, which are distributed over the entire gene but for which the biological significance, if any, remains unclear. Amongst these mutations the Q279* has appeared in two different tumors and introduces a stop codon that eliminates the last six residues from the Gpn3 C-terminal tail. We investigated here if this mutation affects Gpn3 function in human cells. Gpn3R Q279* was unable to fully support RNAPII nuclear localization in MCF-12A epithelial cells. Unexpectedly, Gpn3R Q279* but not the wild-type protein caused the nuclear accumulation of Gpn1-EYFP in HEK293 cells. A sequence analysis revealed that the Q279* mutation generated the formation of a putative PDZ domain binding motif with the consensus sequence D/EXXØ, where Ø corresponds to a hydrophobic amino acid (F278 in Gpn3 Q279*). To test the hypothesis that the mutation Q279* results in the formation of a PDZ domain binding motif, we generated the Gpn3 E280* and F278* mutants, which added or eliminated one amino acid to Gpn3 Q279*, a manipulation expected to eliminate this motif. We also introduced the mutation E276A into Gpn3 Q279* to change the selectivity of the putative PDZ domain binding motif. We observed that the effect we had previously noted on Gpn1-EYFP nuclear accumulation was lost in these mutant versions of Gpn3. We concluded that the cancer-associated Gpn3 Q279* mutant is defective to support nuclear targeting of RNAPII and that the mechanism involves the generation of a new PDZ-binding motif that interrupts the nucleo-cytoplasmic shuttling of the Gpn1/Gpn3 protein complex.

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# Effect of overexpression of dystroglycan on the physiology of HL-60 cells

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Hematopoiesis is the process by which all formed elements of the blood are produced, is a highly regulated mechanism that involves both the participation of various cellular as acellular factors. Hematopoiesis takes place in a highly specialized compartment known as hematopoietic niche. Leukemias are diseases of the hematopoietic system where there is a failure in cell differentiation and characterized by hyperproliferation of cells in early stages of differentiation. It described a decrease or absence of dystroglicans in various solid tumors, where there has been a proteolysis and altered glycosylation. The loss of dystroglycan, which is an adhesion molecule, affects the interactions of the cell with the extracellular matrix, altering proliferation, migration, differentiation and cell polarization. In this work dystroglycan was overexpressed in the cell line HL-60. Overexpression was confirmed by Western blotting and qRT-PCR. The subcellular distribution of the exogenous protein dystroglycan was analyzed and found to be localized in the nucleus and cell membrane of the same shape as the α/β-endogenous dystroglycan. Additionally, the overexpression of dystroglycan decreases cell proliferation and promotes differentiation capacity, which was evident in an increased production of azurophil granules and phagocytic activity. These results demonstrate a key role of dystroglycan in cell proliferation and differentiation in cells HL-60, as observed in the last decade in other cell systems. The altered dystroglycan expression in hematopoietic progenitor cells, which may lead to a loss in the regulation of proliferation and differentiation of these cells in the hematopoietic niche, could be one of the factors involved in the pathophysiology of proliferative diseases such as AML.

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# $Ca^{2+}$ Signal Evoked by Histamine in Normal Human Lung Fibroblasts

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**INTRODUCTION:** Pulmonary fibrosis is a progressive interstitial lung disease characterized by accelerated remodeling of the lung architecture. Several studies have documented mast cells accumulation in the lungs of patients with fibrosis. Mast cells are a major source of histamine. In patients with pulmonary fibrosis, the increase in mast cells number is correlated with increased histamine concentration in the bronchoalveolar lavage fluid. In *in vitro* studies, histamine was able to stimulate lung fibroblast collagen synthesis, migration and proliferation. However, the transduction mechanisms leading histamine to these effects in lung fibroblasts are still unclear. An increase in intracellular free $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) is an important signal for many cellular processes. It has been shown that $Ca^{2+}$ signaling mediate lung fibroblasts proliferation, migration, apoptosis and collagen production. **AIM:** Accordingly, this study aimed to examine the mechanisms underlying histamine-induced increase in ($[Ca^{2+}]_i$) in primary cultures of normal human lung fibroblasts (NHLF) by using conventional imaging microscopy. **METHODS**: Cultured NHLF were loaded with 3μM Fura-2 and $Ca^{2+}$ signals were recorded by microfluorimetric techniques. **RESULTS**: In NHLF, histamine causes a concentration-dependent increase in $[Ca^{2+}]_i$ with a $EC_{50}$ of 2.1 μM. The application of maximal histamine concentration (100μM) to NHLF, elicited a heterogeneous pattern of $Ca^{2+}$ signals even in cells from the same microscope field: a) a single $Ca^{2+}$ spike that could be followed by b) $Ca^{2+}$ oscillations, c) a sustained $Ca^{2+}$ plateau or d) a plateau overlapped by $Ca^{2+}$ oscillations. The specific histamine H1 and H2 receptors agonists, N-methylhistaprodifen (10μM) and amthamine (10μM), mimicked histamine effects. Few changes in the intracellular $Ca^{2+}$ concentration were observed even at high doses of (R) (-)-α-methylhistamine (10μM) and clozapine (10μM), selective agonists for H3 and H4, respectively. Pharmacological manipulation revealed that histamine activates a $Ca^{2+}$ signal through $Ca^{2+}$ release from intracellular stores mediated by phospholipase C (PLC), $Ca^{2+}$ release from inositol 1,4,5-trisphosphate receptors ($IP_3Rs$) and $Ca^{2+}$ influx via a store-operated pathway. In cells showing a plateau or $Ca^{2+}$oscillations, removal of extracellular $Ca^{2+}$ or using a store-operated channel inhibitor, 2APB, caused an immediate decrease of $Ca^{2+}$ levels to the baseline. **CONCLUSIONS**: Histamine increases $[Ca^{2+}]_i$ in NHLF through the activation of H1 and H2 receptors, $Ca^{2+}$ release from intracellular stores and, $Ca^{2+}$ entry to store operated channels.

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# Evaluation of treatment of second degree burns with adipose derived mesenchymal stem cell using human radiosterilized amnion and pig skin as scaffolds.

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The American Burn Association reported 486,000 patients receive medical treatment related to burns. Second and third-degree burn patients require autologous skin grafting; however, when more than 50% of the total body surface is affected, availability of healthy skin for autologous transplantation is limited. In these patients is possible to obtain adipose tissue and isolate Adipose-derived mesenchymal stem cells (ADMSCs). These cells have multiple therapeutic effects, not only through the differentiation and incorporation to damaged tissue but also by promotion of angiogenesis (important during neo-tissue formation), the release to interleukin-10 (IL-10), an anti-inflammatory cytokine involved in regenerative processes. Radio-sterilized human amnion (RHA) and pig skin (RPS) have been used as temporal dressings for burn patients. These biological dressings prevents heat and water loss from the wound surface, diminishing pain sensation and acts as a barrier against bacterial contamination. However it has never been studied if they are good scaffolds to support the growth of ADMSC and be useful for delivery of these cells to burn sites and the effect of this in the scarring mechanisms. **Methods**. ADMSC were obtained from aesthetic surgeries undergoing elective liposuction. First passage cells were analyzed to detect CD90+, CD73+, CD34- and CD45- markers by flow cytometry. Cells were differentiated to chondrocytes, osteocytes and adipocytes. Differentiation was confirmed performing immunofluorescence for Collagen II and Runx2; oil red staining was also tested. ADMSC were seeded onto RHA and RPS; cell viability, proliferation, and cytokines secretion was analyzed. Second-degree burns were performed in NU/NU mice; analyzed conditions were: control, RHA, RPS, RHA+ADMSC and RPS+ADMSC treatment. Outcome measurements include percent area of wound closure by photographs, wound assessment and extracellular matrix deposition using histological staining(H&E, Masson and Herovici) at 7 and 14 post-burn. **Results.** ADMSC were successfully obtained, they expressed CD90+ and CD73+ markers. Cells were capable to differentiate to chondrocytes, osteocytes and adipocytes according to protein expression. Cells seeded onto RHA and RPS showed more than 95% of viability. Proliferation of ADMSCs cultured onto RHA was increased when compared to those cultured onto RPS. In both scaffolds, these cells release IL-10; however, cells grown onto RPS secrete more IL-1β. Our preliminary results in murine burn models showed that treatment with scaffolds plus ADMSC present more collagen deposition after 7 days of treatment and a better repithelization at 14 days. Remarkably, using these treatments we were able to observe formation of skin appendages. **Conclusion.** RHA and RPS are suitable scaffolds for the growth of ADMSC. These biological dressings in combination with ADMSCs induce a better skin recovery in second-degree burns after 7 and 14 day of treatment, nevertheless the 14-day observation may not be sufficient to evaluate scarring mechanisms.

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# The Human Papillomavirus type 16 E5 and Ras Oncoproteins control cell differentiation to favor transformation.

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**Introduction:** The E5 oncorpotein from HPV16 (HPV16-E5) modulates the keratinocyte differentiation, which is important to allow the progression of viral cycle for generation of viral particles, or cellular transformation in persistent infections when HPV16 ***E6*** and ***E7*** oncogenes become over-express. Also, the E5 oncoprotein synergized with the EGFR to control the cell cycle through the modulation of $p27^{Kip1}$ levels. In addition, the Ras protein is part of the EGFR signaling pathway and together with E5 are regulating cell division and differentiation.
**Objective.** To characterize the molecular mechanism through which HPV16-E5 and Ras modulate cell differentiation to allow progression of viral cycle, or cellular transformation.
**Methods.** Cell lines derived from human (HaCat) and mouse (NIH-3T3) origins were transfected with HPV16-***E5***, alone or combined with ***Ha-ras*** oncogene. Cell transformation was measured by growth curves, FACS cell cycle analysis and colony formation in soft agar, in the presence or absence of EGF. To evaluate the degree of differentiation, normal and E5-expressing cells were grown in 0.2% FBS and high $CaCl_2$ concentration for different periods of time. Levels of Cytokeratin 14 (CK-14, undifferentiated cells) and CK-1 (differentiated keratinocytes) and other differentiation protein markers were evaluated by Western blot.
**Results.** We observed a non-additive activity between Ras and E5 in cells expressing both oncogenes, as a reduction in proliferation and transformation was detected. On the other hand, when cell differentiation was evaluated by the cytokeratins pattern, it was observed that E5-expressing cells presented low levels of cytokeratins as compared to HaCat control cells. When cells were induced to differentiation with calcium, the CK-14 was reduced in HaCat cells, but it was absent in E5-expressing cells as well as the CK-1. Experiments are in progression to evaluate the role of Ras and E5 together during keratinocytes differentiation.
**Conclusions.** The activities of HPV16-E5 and Ras are not additive during cell transformation process as it is observed with HPV16-E7 and Ras. The null or low levels of cytokeratins in E5-expressing cells suggests that the differentiation process has been arrested to maintain the viral cycle in the cells, but if this process persist the cell could loss control and become transform.

# Effect of hydrogen sulfide on yeast autophagy

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Autophagy is a self-digesting process in which the cell degrades its own cytoplasmic material through the formation of vesicles (autophagosomes) engulfing the target material and a subsequent fusion with lysosomes (autolysosome) to degrade and recycle it. Hence, this process renews cytoplasmic machinery and is essential for nutrients and energy replenishment during stress conditions such as starvation. Autophagy inhibition has been associated with premature aging and neurodegenerative diseases. Moreover, it has been shown that restriction of sulfur amino acids methionine and cysteine extends longevity and induces autophagy. One of the major non-desired subproducts in fermentation industry due to S-containing amino acid catabolism is the well-known toxic gas hydrogen sulfide ($H_2S$). Also described as the third gasotransmitter, $H_2S$ is physiologically generated bycystathionine$\gamma$-lyase (CSE) and cystathionine β-synthase (CBS) involved in cysteine and methionine assimilatory pathway. In mammals, it has been demonstrated $H_2S$ angiogenic, antioxidant and antiapoptotic properties useful in age-associated diseases. Recent studies in yeast show that $H_2S$ production via transsulfuration pathway is increased upon dietary restrictionyielding to extended chronological longevity, which could be regulated by autophagy. The aim of this work is to assess whether $H_2S$ is an autophagy-modulator molecule in the budding yeast *Saccharomyces cerevisiae*. As autophagosome biogenesis upon nitrogen starvation requires Atg8 release and cleavage from the conjugated Atg8-PE localized to both the outer and inner autophagosome membrane we detected this breakdown by fusing GFP to Atg8. We first induced autophagy by amino acid deprivation and set up the best conditions to monitor and quantify the autophagy flux by a GFP-Atg8 assay using immunoblotting. In this direction, the yeast was exposed to a $H_2S$ donor molecule at non-lethal concentrations at different times. Furthermore, to observe autophagosome formation fluorescence microscopy will be performed. Additionally, different mutants of the methionine and cysteine biosynthesis pathway will be exposed to $H_2S$ to determine what step could be responsible for autophagy induction. In summary, it is remarkable that our findings will provide new insights into the potential biological function of $H_2S$ in yeast.

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# Analysis of of UAP56 homologue (RNA helicase) participation in the mRNA export of C6/36 cell line derived from *Aedes albopictus*

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In several organisms has been demonstrated that splicing of pre-messenger RNA, processing and export of mRNA are highly coordinated *in vivo*. Capping and splicing are important for the recruitment of the transcription-export (TREX) complex which is highly conserved and is essential for mRNA export. In *Saccharomyces cerevisiae, Caenorhabditis elegans* and *Drosophila melanogaster* is well established that TREX elements as UAP56 (BAT1 or Sub2p), NXF1 (Mex67p) and p15 are essential factors for mRNA export and inactivation or depletion of these proteins results in a significant accumulation of polyadenylated RNA within the nucleoplasm. UAP56 is a member of the DEAD-box family of splicing factors, which have ATPase and RNA helicase motifs, and also interacts with the mRNA export factor ALY, mediating the first ATP-dependent step of spliceosome assembly. UAP56 associates with proteins of the THO complex (Suppressors of the transcriptional defects of hpr1 delta by overexpression) which are transcription elongation factors included in the TREX complex, and also interacts with splicing machinery to form the exon-junction complex. In this work we use as model the C6/36HT cell line, derived from *Aedes albopictus* (Aealb), to study the expression of the components of the TREX complex, including a RNA helicase homologue (UAP56), and its participation in the mechanism of mRNA export. To reach this goal we designed oligonucleotides based on *Ae. aegypti* genome, in order to study the expression of key molecules. We found UAP56 expression both, as messenger and protein. It is located in the cytoplasm and by immunoprecipitation assays and mass spectrometry we analyze the UAP56 partners forming the AealbTREX complex.

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# Estrogen-mediated down-regulation of E-cadherin in breast cancer cells is mediated by c-Src and promotes cell migration and invasion.

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It is well known that E-cadherin plays a crucial role in breast tumor suppression and that estrogens cause down-regulation of E-cadherin levels in both normal and tumorigenic breast epithelial cells (*Cancer Res* 63:5203, 2003). We have recently proposed a novel pathway that shows estradiol induces tight junction (TJ) disruption, epithelial to mesenchymal transition (EMT) and cell migration throughout cSrc activation (*Horm Cancer 5:161, 2014*). Since down-regulation of E-cadherin is also related to cell migration and invasion, we decided to study the effect of estrogen-induced cSrc activation on the expression and localization of E-cadherin, and the subsequent effects on cell migration and invasion using two ER-positive breast cancer cell lines, MCF-7 and T47D.

We have been able to demonstrate specific interactions between $^{416}$Tyr-Src/$^{861}$Tyr-FAK (Focal Adhesion Kinase) and $^{861}$Tyr-FAK/E-Cadherin, by co-immunoprecipitation, after the incubation with 1 nM of estradiol ($E_2$). This effect was partially precluded when MCF-7 cells were treated either with the antiestrogen ICI 182,780 (ICI; 0.1 μM) or the selective c-Src inhibitor PP2 (5 μM). Incubation with $E_2$ led to a marked reduction in E-cadherin mRNA levels, whereas no decline occurred when either ICI or PP2 were used. Western blot and immunofluorescence studies confirmed the reduced E-cadherin protein levels and its aberrant localization, respectively. E-cadherin redistribution is blocked by the inhibitory effects of ICI or PP2. Finally, wound-healing and Transwell cell invasion assays confirm the effects of the $E_2$-induced E-cadherin down-regulation on increased cell migration and invasion.

We propose that the $E_2$-induced de-regulation of E-cadherin can be associated to cell migration and invasion changes, through activation of c-Src/FAK, which can contribute to metastasis and/or development of human breast cancer.

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# *PKCz-CDP-FIH-HIF status at early stages of renal carcinogenesis and tumors induced by ferric nitrilotriacetate*

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Renal cell carcinoma (RCC) has a high mortality since, among other issues, it is asymptomatic and there is no specific early markers, so initial diagnosis frequently occurs at advanced or metastatic stages, which makes almost impossible the study of its initial phases. Ferric nitrilotriacetate (FeNTA)-induced RCC model is a useful tool to analyze molecular events at different states of the carcinogenesis process *in vivo*. Previously, we reported that RCC tumors produced in rats by FeNTA are histologically identical to human neoplasm, and identified one and two months of treatment as different early stages of carcinogenesis.

Increased levels of PKCz have been reported in human RCC cell lines, coinciding with a rise of HIF and a decrease of FIH; this relationship seems to be mediated by the effect of PKCz on the transcriptional repressor CDP which regulates FIH gene expression; nevertheless, the status of all these molecules is unknown in human tumors, and the pathway's behavior has not been studied in the different phases of FeNTA model.

In the present work augmented PKCz renal levels were found after one and two months of FeNTA exposition as well as in the experimental tumors. On the other hand, analysis at one month showed no changes in the amounts of HIF-1a and 2a, increased protein and RNAm levels of both isoforms were observed at two months of treatment and in neoplasm, which coincided with a PKCz rise. Accordingly, at two months and in tumor tissue FIH protein levels decreased, however no changes in its mRNA were found.

In conclusion, PKCz-CDP-FIH-HIF pathway behavior evolves as carcinogenesis process advances, and our results suggest its participation since early stages of cancer development and in tumor maintenance, but not at initial phases.

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# Invadopodia formation and matrix metalloprotease secretion during epithelial-mesenchymal transition induced by leptin in MCF10A epitelial cells

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**Introduction:** Cell invasion is a key process during tumor progression where cancer cells exhibit specialized structures in degrading the extracellular matrix and invade through ECM. The invadopodia are structures rich in F-actin called actin puncta and it is regulated by some proteins as cortactin, Tsk-5 and recently it has been described Hic-5. Additionally, it has been observed an increase in MMPs secretion, particularly in MMP-2 and MMP-9 secretion, these events can promote local invasion and consequently tumor progression to a metastatic phenotype. Recent reports have described an association between obesity and breast cancer, this effect can be attributed to a high concentrations of leptin synthesized locally by adipose tissue in mammary glands and leptin now is recognized as an inducer of EMT in MCF10A cells. **Objective:** To evaluate the effect of leptin in invadopodia formation, Hic-5 expression and MMP-2 and MMP-9 secretion. **Methods:** We employed a protocol stimulation based in time-course and dose-response assays with leptin in MCF10A cell cultures; MMP-2 and MMP-9 secretion was determinated by zymography, Hic-5 expression by Immunofluorescense, and finally invadopodia formation assays were performed. **Results:** Leptin induces an increase in MMP-2 and MMP-9 secretion in a time-dose dependent and an increase in invadopodia formation as well as Hic-5 expression in MCF10A cell line. **Conclusion:** Leptin promotes cell invasion by invadopodia formation, Hic-5 expression and MMP-2 and MMP-9 secretion in MCF10A cells.

**Keywords:** EMT, invasion, invadopodia, MMP-2, MMP-9, leptin

# Estudio de la fosfolipasa D en los mecanismos de migración e invasión inducidos por ácido linoleico en células de cáncer de mama MDA-MB-231

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Abstract

Epidemiological and animal studies suggest that high intake levels of fatty acids (FA) in the diet are associated with an increased risk of developing breast cancer. Linoleic acid (LA) is an essential element and the principal FA polyunsaturated (PUFA) in the Western diet; It is able to induce inflammatory responses are inappropriate, contributing to several chronic diseases, including cancer. In breast cancer cells, the AL induces proliferation and migration, however, the molecular mechanism by which modulate migration has not been well defined.

Previous studies have shown that the way in which FAs act as extracellular ligands to induce biological effects is activating G protein-coupled receptors (GPCRs), specifically for the AL has been described the GPR40 and GPR120 receptors, which have been reported to be expressed in MCF7 and MDA-MB-231 breast cancer cells. Furthermore, studies in various cell lines indicate that FA is able to use the epidermal growth factor receptor (EGFR) as an intermediary in the signaling, in a process described as transactivation of GPCR to the EGFR, however, the mechanisms underlying to stimulation with AL they not have been studied well.

A protein associated with migration processes during tumor progression is phospholipase D (PLD). PLD is an enzyme that has two isoforms, PLD1 and PLD2, its main function is to phospholipids hydrolyze membrane, specifically phosphatidylcholine, generating the second messenger phosphatidic acid (PA), involved in different cell signaling pathways such as proliferation, migration and cell invasion.

In breast cancer cells, stimulation with AL induces proliferation and migration, whereas in cells non-tumorigenic breast epithelium induce an epithelial-mesenchymal transition type process. In the present work, our results demonstrate that the AL induces activation of PLD in mammary tumor cells MDA-MB-231, as well as activation of the transcription factor NFkB. Furthermore, activation of NFkB, and the processes of migration and invasion cell induced by AL is dependent on GPR40 and GPR120, EGFR, and activity of both isoforms of PLD receptors.

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# Characterization of protein complexes associated to the phosphatidylinositol 3-kinase during *Phaseolus vulgaris*-rhizobia interaction

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*Phaseolus vulgaris* (common bean) as other legumes is able to establish symbiotic relationships with bacteria of the genus *Rhizobium.* The establishment of this interaction requires a molecular exchange of signal molecules between the two symbionts, resulting in the formation of nitrogen fixing nodules. The symbiotic nodule development is a complex process that includes vesicular trafficking and cell differentiation, among others (1,4). Phosphatidylinositol 3-phosphate (PI3P) is a phosphoinositide that is present at very low levels in plant cells (2). It is synthesized by phosphatidylinositol 3-kinase class III (PI3KC3), encoded by the only *PI3K* gene reported in plants. PI3KC3 coordinates processes involved in autophagy, vesicular trafficking and endocytosis, among others (3). Hitherto, in yeast and animals several PI3KC3 associated proteins, have been reported (5). However in plants similar protein complexes have not yet been completely described. Herein, we have focused on the identification and characterization of *P. vulgaris* PI3KC3 associated protein complexes during symbiotic process. A Tandem Affinity Purification (TAP) approach is being used to do this, utilizing an adequate construct that allowed us to obtain a high level of expression of PI3K under its own promoter. Currently, bean plants with transgenic roots expressing such construction have been obtained. These transgenic roots were inoculated with *Rhizobium tropici* to get nitrogen-fixing nodules, from which the protein complexes of interest are produced for further purification and characterization. Experiments are in progress to obtain enough protein concentrations for their identification by LC-MSMS.

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# The effect of hyperglycemia on the expression of stress proteins of endoplasmic reticulum and vascular smooth muscle cell (VSMC) migration from normal rats

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Diabetes mellitus (DM) is a chronic degenerative disease characterized by high glucose levels on blood, which is the leading cause of micro and macrovascular changes observed in this disease.

At cellular level DM promotes the misfolding of proteins in the endoplasmic reticulum (ER), a condition defined as ER stress. ER stress is characterized by an increase on the expression of some proteins, unfolded protein response (UPR), that promotes the decrease of the unfolded proteins translation and the increase in the expression of folding proteins or apoptosis when the damage is too great.

Since research efforts on macrovascular disease secondary to DM are focused on pathological changes on endothelial cells, we are interested in evaluating the changes that occur at the level of VSMC as it has been demonstrated that they play a fundamental role in the pathophysiological process.

On this research we obtained primary smooth muscle cell cultures from the thoracic aorta of normal rats by means of explant culture technique. The cells were characterized by immunofluorescence against muscle alpha- actin and myosin and they were used on experiments were the cells were exposed to high glucose concentrations (15 mM) where we analyzed: proteins related to endoplasmic reticulum stress, expression of $Ca^{+2}$ ATPase of the ER (SERCA2), cellular migration through the method of wound healing assay and the usage of the *Boyden* camera. Our results show that muscle cells exposed to 15 mM of glucose for 48 hours do not exhibit any changes on cell migration or in the expression of ATF-6 and XBP-1 proteins (analyzed by western blot), that we used as stress markers. However cells exposed for one week to 15 mM glucose showed an increase on cell migration as well as an increase on the expression of stress markers, which leads us to believe that these cells have a phenotype that is similar to those from diabetic rats. On the other hand the expression of SERCA2, analyzed by inmunoblot, seems to increase which suggests that the cells that are exposed to hyperglycemia over express SERCA2 to compensate for the damage on the ER being that the activity of this ATPase is fundamental to maintain a proper cell function. Overall these results show that the expression of smooth muscle cells to high glucose concentrations simulates the process that takes place in cells from diabetic animals, which allows us to have a study model of diabetes without inducing animals with drugs that produce severe side effects.Supported by Conacyt to RET: 223350.

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# Non-Classical Effects of Androgens in Muscle Cells

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Recently, it has been found that the effects of testosterone can be mediated by two different mechanisms of action, the classical pathway and non-classical action of androgens. In the "classical pathway" testosterone binds to its receptor cytosolic androgen (AR), a 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 proposed that testosterone binds to receptors on the plasma membrane and induce the activation of intracellular signaling cascades mediated by activation of ERK1/2. 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. To determine the molecular mechanisms of non-classical actions of testosterone (Tes) and its active metabolite dihydrotestosterone (DHT) we evaluate the activation of ERK1/2 in skeletal muscle cells C2C12. We found that Tes and DHT induce rapid ERK1/2 phosphorylation (0-30 min), effect that is depending of time and concentration. By using flutamide, an antagonist of cytosolic ARs, we found that both androgens induce activation of ERK by a mechanism independent of cytosolic ARs.

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# Isolation, characterization and neural differentiation of Wharton´s Jelly Mesenchymal Stem Cells from Human Umbilical Cord.

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*Key words: Mesenchymal stem cells, Wharton´s jelly, characterization, neural differentiation.*

**Introduction.** The Wharton´s jelly mesenchymal stem cells (hWJMSC) are a particular group of birth-associated tissue stem cells isolated from the matrix of human umbilical cord. These cells are characterized by the ability to adhere to plastic culture flask, express surface markers such as CD13, CD44, CD73, CD90 and CD105, lack expression of CD45, CD34, CD31, CD14, CD11b, CD19, and differentiate into osteoblast, chondrocytes and adipocytes, according to the Institute of Stem Cell Therapy (ISCT) criteria (1). In the last years the research of these cells has increased, due to the high replicative, clonogenic potential, ability to differentiated in mesenchymal and non-mesenchymal cell linages, immunosuppressive potential and the easy and non-ethical considerations in the isolated method,(2). The umbilical cord is considered a waste tissue, positioning hWJMSC as candidates for the therapeutically repair of tissues, and can be used as a cellular model for the understanding of the mechanisms involved in differentiation process and diseases (3). Different protocols describing the isolation, characterization and differentiation exist, however they are inconclusive and difficult to achieve (4). The main purpose of this study is to obtain an enriched population of hWJMSC through an easy isolation protocol, purify them by cell sorting, and characterize them according to ISCT guidelines. We also compared different neural induction protocols and selected the most efficient.

**Materials and methods.** The Wharton Jelly Mesenchymal Stem cells were isolated from human umbilical cord matrix, the perivascular tissue was minced mechanically and was seeded in CHANG medium supplemented with penicillin, streptomycin and fungizone, fresh medium was replaced every 3$^{rd}$ day. At day 15, mesenchymal stromal cell colonies were visible. Negative selection of endothelial and/or hematopoietic CD31, CD34 and CD45 cell markers were employed to sort the matrix cells by Fluorescence Activated Cell Sorting (FACS). The enriched cells were proliferated in CHANG medium and expanded in p60 flasks. Confluent cultures were characterized for the expression of $CD13^+$, $CD31^-$, $CD34^-$, $CD44^+$, $CD45^-$, $CD73^+$, $CD90^+$ and $CD105^+$ by flow cytometry. Differentiation analysis was carried out with Adipogenic Differentiation Media (Stem Cell Technologies) to demonstrate the ability of hWJMSC to differentiate into adipocytes, and was confirmed under microscopic analysis using Oil Red O staining (Sigma-Aldrich. To induce neural differentiation, hWJMSC were cultured at $1x10^5$ in CHANG medium. We tested diverse neural induction protocols: 1) using forskolin 10µM in DMEM 1%FBS for 14 days. 2) By preinduction with bFGF 10ng/mL in DMEM 2% for 3 days following induction with forskolin 10µM in DMEM 1% for 14 days. 3) Using different combined protocols with B27 supplement, BDNF 10ng/mL, retinoic acid 20uM, valproic acid 2mM, BHA 200µM, with or without forskolin and bFGF, according to previous reported protocols. Neural differentiation efficiency was assessed by mRNA expression of stem and neural markers as Nestin, SOX2, Tubβ3, MAP2, OLIG2 and GFAP by RT-PCR and by immunostaining with Nestin, SOX2 and Tubβ3.

**Results.** The isolation method of hWJMSC proved to be useful and easy; the typically fibroblastic spindle cells morphology was observed. Approximately 40-45% of all matrix cell population did not express CD31, CD34 and CD45 markers, but express CD13,

CD44, CD73, CD90 and CD105. After FACS selection, we obtain an approximately 80-85% enriched culture with the interest phenotype. The hWJMSC have the ability to differentiate into adipogenic linage, because the oil-red O staining showed positive staining lipid drops. The neural induction protocols demonstrated a major number of neural differentiated morphology in the protocol with preinduction with bFGF 10ng/ml for 3 days and subsequent induction with forskolin 10µM during 14 days. At mRNA level, we found expression of Nestin, SOX2, Tubβ3 and MAP2, but absence expression of OLIG2, and GFAP. The immunostaining showed the expression of Nestin, SOX2 and Tubβ3 in untreated cells. After differentiation, the cells showed an increase staining of Tubβ3 in cells with neural morphology, and no or faint expression of Nestin and SOX2 markers.

**Conclusion.** The human Wharton Jelly Mesenchymal Stem cells isolated from perivascular zone of umbilical cord by explant and cell sorting show the phenotype reported for ISCT criteria and the ability to differentiate into two different cell linages, and demonstrate the plasticity potential of hWJMSCs. The most efficiency neural induction protocol was bFGF with Forskolin in DMEM 1% FBS, and showed the expression of Nestin, SOX2, and Tubβ3 at mRNA and protein level.

**Acknowledgments.** This work was supported by INPer grant 3140-21202-01-15.

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# Apigenin regulates the expression of inflammatory kinases induced by LPS of *Porphyromonas gingivalis* in cell line H9c2.

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Oral bacteria have virulence factors which cause rapid colonization that often result in Periodontitis, however, it is known that these infectious agents are associated with systemic diseases, being endocarditis and cardiovascular risk main conditions. This study aims to investigate the effects of Apigenin (flavonoid) in the expression of inflammatory cytokines induced by Lipopolysaccharide of *Porphyromonas gingivalis* (predominant bacteria in periodontal disease) in H9c2 cells (cardiomyocytes). To rule a cytotoxic effect of flavonoid was performed cell viability assay.By Western blotting it was evaluated the expression of p38 kinase, ERK (1/2) and JNK induced by PAMP, whereby time curves were performed in the presence of LPS (1 g / mL) .Once demostrated the effects of LPS in cells , it was evaluated by a test dose response effect of Apigenin in regulating proinflammatory kinases mentioned above. In order to assess whether Apigenin acts on transcription RT-PCR was performed with the mentioned dose-response protocol. Apigenin did not shows cytotoxic effect on the cell line ; regarding the induction of proinflammatory kinases by LPS, it was observed that increasing time of PAMP, the expression of these kinases considerably increases compared to baseline, and in presence of Apigenin it is declines expression dose-dependent manner. In conclusion Apigenin can regulate the expression of inflammatory kinases LPS-induced which contribute to future treatments for cardiovascular diseases.

This proyect (IN201816) was supported by PAPIIT (Programa de apoyo a proyectos de investigación e innovación tecnológica PAPIIT).**Effects of p-**

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# Effects of p-Chloroamphetamine (pCA) on Follicular Development and Apoptosis in Prepuberal Rat.

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Amphetamine derivatives, such as pCA, has been used in experimental studies in animals due it induces neurotoxic effects in the serotonergic system. Serotonin is a biogenic amine present in the ovary. Its possible that serotonin receptor, subtypes 5-$HT_{2A\text{-}B\text{-}C}$ and 5-$HT_7$, in granulosa and cumulus cells have a role in estradiol secretion, which in turn acts as a survival factor for granulosa cells. Several drugs used in the treatment of psychological and physical disorders enhance or inhibit serotonergic activity; nevertheless, their effects in reproduction are not much known. The aim of this study was to analyse the effects of pCA on estradiol concentration, follicular development and apoptosis in granulose cells.

Thirty-day-old rats of the CIIZ-V strain were treated intraperitoneally with pCA (Sigma Chemical Co., St. Louis, MO, USA; dissolved in 0.1% NaCl) 10 mg/Kg of body weight. Control rats were injected with saline solution (0.1%) (VH). An untreated group was also used as a control (TA). All animals were killed 120 h after the treatment. The serum concentration of estradiol was measured by radioimmunoassay. Follicular diameter was measured and classified in ovarian tissue sections; presence of apoptotic cells was evaluated (TUNEL assay) in the same tissue sections.
Serum concentration of estradiol was lower in the animals treated with pCA (TA: 23.8 ± 2.3; VH: 21.7 ± 2.2 vs. pCA: 11.0 ± 1.0, $p<0.05$). The number of total follicles and by class was not modified, although the number of follicles in the distinct classes was higher in the animals treated with pCA: class 1 (<100 □m of diameter) (TA: 2.66 ± 0.33 vs. pCA: 6.6 ± 2.13, $p<0.05$); class 2 (101-199 □m of diameter) (TA: 16.3 ± 1.20; VH: 14.8 ± 3.8 vs. pCA: 26.8 ± 1.59, $p<0.05$) and class 3 (200-349 □m of diameter) (TA: 11.33 ± 0.88; VH: 12.2 ± 2.59 vs. pCA: 30.2 ± 4.09, $p<0.05$) with apoptosis was increased.

The increased presence of follicles with apoptotic granulosa cells, in animals treated with pCA, could be related with the low concentrations of estradiol detected in the same animals. Our results reinforces the idea of the negative effects of amphetamines in female reproduction.

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# Native type I collagen induces an epithelial to mesenchymal transition process in mammary epithelial cells MCF10A

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The extracellular matrix (ECM) is a complex network of insoluble macromolecules which form the basement membrane and interstitial matrix, and regulate various biological processes. In particular, the interstitial matrix is formed by stromal cells and is composed by different types of fibrillar collagen, the most abundant collagen type I (col-I), which maintains interaction with cell surface receptors like integrins and discoidin domain receptors (DDRs). DDR2 is activated by collagen type I, II, III and collagen X. The overexpression of this receptor has been strongly correlated with lymph node metastasis and poor patient survival, promoting processes such as survival, growth, migration, invasion and epithelial mesenchymal transition (EMT) in different cell types. EMT is a biological process that allows epithelial cells to acquire a mesenchymal phenotype characterized by loss of epithelial markers and gain of mesenchymal markers promoting the metastatic process. However, the mechanism through which the DDR2 participates during EMT in mammary epithelial cells under the stimulation with collagen type I, has not been completely elucidated. Here, we demonstrate that treatment with collagen type I native promotes an expression increase of N-cadherin and vimentin, a raise in the secretion of MMP-2 and MMP-9, destabilizes adherent junctions through the decrease of expression of E -cadherin. Furthermore, collagen type I promotes cell migration and phosphorylation of FAK and Src kinases. Also, under this stimulus the activation of NFκB and E-box associated transcription factors was induced. In conclusion, our results suggest strongly that native col-I participates during EMT process of EMT in cells MCF10A.

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# Regulatory Mechanisms of Tumor Endothelium Marker 4 (ARHGEF17)

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Angiogenesis, which normally occurs in processes such as wound healing, is the formation of new blood vessels from preexisting ones. In cancer, angiogenesis promotes growth and metastasis of tumor cells. Therefore, inhibition of tumor angiogenesis is the basis for antineoplastic therapy. From this perspective, proteins with exacerbated expression or activity in endothelium or tumor stroma represent potential drug targets. RhoGEFs are involved in the remodeling of the actin cytoskeleton, process related to cell migration and angiogenesis. The RhoGEF known as ARHGEF17 (or TEM4, tumor endothelium marker 4) was found overexpressed in tumor endothelium of colon cancer (Croix, 1999, Science.289:197). Initial studies with a partial clone of ARHGEF17 indicated their specificity for RhoA and suggested the existence of intramolecular interactions ( Rumenap 2002, Biochem J. 366,721). Our long-term goal is to understand the role of ARHGEF17 in tumor angiogenesis. Here we demonstrate that in endothelial cells, this RhoGEF is activated in response to lung carcinoma (LAP0297) tumor cell conditioned media and the G protein-coupled receptor responsive to lysophosphatidic acid. In order to understand their regulatory mechanisms we generated constructs including the catalytic module anchored to the membrane, which itself functions as a constitutively active variant of ARHGEF17. This construct strongly activated RhoA and initiated a signaling cascade that stimulated the GTPase Rac. Furthermore, we demonstrated the existence of inhibitory intramolecular interactions, involving the association of amino and carboxyl regions with the DH-PH catalytic module. Finally, the factors secreted by tumor cells favor the formation of dimeric RhoGEFs, which correlated with the activation process. The dynamic activation of ARHGEF17 in endothelial cells stimulated by tumor cell-secreted factors suggests its involvement in tumor induced angiogenesis.

# The effect of hydrogen sulfide in the transcriptome of *Saccharomyces cerevisiae.*

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Hydrogen sulfide ($H_2S$) is a newly identified member of the family of gasotransmitters and is produced by every living being on this planet (microorganisms, plants and animals). Indeed, there is evidence that suggest that $H_2S$ was involved in the origin of life and in the earliest development of eukaryotic cells. The cellular effects of hydrogen sulfide have been linked to many physiological systems as the cardiovascular and central nervous systems.

Consequently abnormal hydrogen sulfide metabolism is implicated in many diseases including hypertension, heart disease, atherosclerosis and inflammation. In lower eukaryotes as the yeast *Saccharomyces cerevisiae* the $H_2S$ is a sub product of fermentation, however the biological role of endogenous production in yeast has never been studied. Yeast has three enzymes that produce $H_2S$ the cystathionine-β-synthase, the cystathionine-γ-lyase and the heterodimer sulfite reductasa, coded by the genes *CYS4*, *CYS3* and *MET5-MET10* respectively.

In mammals $H_2S$ mediates its effects through a protein posttranslational modification but the effect at transcriptional level needs to be elucidated. The goal of this work is determine the role of $H_2S$ at transcriptomic level in the yeast *S. cerevisiae* using RNA seq.

We constructed singles, double and triple mutants of the three enzymes involved in $H_2S$ production and determine $H_2S$ production in different growth conditions (different carbon source). Additionally we obtained the non-lethal concentration of $H_2S$ that is 20 μM and is very similar to endogenous concentration of $H_2S$. We realized growth kinetics with the wild type using GYY4137 (a slow donor of $H_2S$) in different concentrations in order to obtained the maximum concentration that does not affect the growth rate. Finally we used 1mM of GYY4137 to analyzed the expression by RNAseq.

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# Role of TOR signaling pathway during *Azospirillum-Arabidopsis* interaction

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*Key words: Azospirillum, Arabidopsis, plant-microorganism interaction, target of rapamicyn*

The genus *Azospirillum* has the capacity to improve plant growth. The mechanism used by these bacteria to promoting plant growth is based in the production of phytohormones, leading to the modification of the architecture of the root system. However, aspects biochemical and molecular during the interaction are unknown. TOR (target of rapamicyn) is a protein related to the family of phosphatidylinositol 3-kinase-related kinases, initially identified in yeast. TOR protein is part of a signaling network that regulates cell growth and proliferation. The deregulation of the activity of this protein in animals, leads to the generation of diseases such as cancer, diabetes and others. The mutation of the gene encoding the TOR protein is lethal to plants, thus pointing to its role in plant development. Several studies have demonstrated the importance of this protein in regulating the development of the root system. Mutant plants with RNAi or treated with inhibitors have a lower development and growth. We decided to analyze the role of this protein in modifying the root system of *Arabidopsis* induced by *Azospirillum*. *Arabidopsis thaliana* (Col 0) seedlings were inoculated with different concentrations of *Azospirillum brasilense* Sp245. The inoculation reduced root length and induces lateral root formation, root hairs and increase in fresh weight. In addition, *tor-es* plants were treated with different concentrations of estradiol to inhibit TOR protein. Estradiol reduced root length at 0.1 µM. Studies are in progress to study the effect of *Azospirillum* on TOR pathway in *Arabidopsis.*

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# Generation of Reporter Lines for the Study of MPK6 Role in the Development of Root Hairs in *Arabidopsis thaliana*

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The Root Hairs (RH) are a model often used to try to understand the mechanisms of cell differentiation and expansion. In the development program of RHs is involved different genetic, molecular and cellular processes, driving activation of signaling pathways in which reactive oxygen species (ROS) and calcium ($Ca^{2+}$) gradients, play important roles to determine the root epithelial cells (trichoblast) which give rise to RH. Recent work has shown that MAPKs (Mitogen Activated Protein Kinases) are one of the components of the signaling pathway controlling the formation of trichoblast. Particularly, has been reported that a null mutant for the gene MPK6 (*mpk6*) has an exacerbated formation and elongation of RHs (1). However, little is known about the function of this kinase in the regulation of this development program. So, this research was focused in generate experimental tools (reporter plant lines) that eventually be can used to obtain knowledge about of the function that MPK6 has in RH determination and growth. For this, we introgressed by controlled crosses the mutation on *MPK6* gene to the reporter lines of cell identity GL2::GUS (atrichoblast marker; 2) and EXP7::GUS (trichoblast marker; 3) also to reporter lines that allow monitoring changes in concentrations of $Ca^{2+}$ (CASE12:GFP; 4) and ROS (HYPER:YFP; 5). The preliminary results of our comparative analysis shows that the highest number of root hairs that typifies the *mpk6* mutant is associated with a greater number of cortical cells, but not to a loss of identity of epidermal cells that give rise to trichoblasts. The analysis of variations in the levels of ROS and Ca2+ during growth of RHs is ongoing and will be discussed at the congress.

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# c-Src inhibitor induces cell migration and invasion through the estrogen-mediated pathway in a Triple-Negative Breast Cancer cell line

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Triple-negative breast cancers (TNBC) are characterized by the lack of expression of estrogen receptors (ER), progesterone receptor (PR), and type 2 Epidermal Growth Factor Receptor (HER2). Because of the absence of targeted therapies, TNBC patients are managed with standard chemotherapy; however, such treatment leaves them associated with a high rate of local and systemic relapse and is not curative for any patient with metastatic disease. One potential therapeutic target against breast cancer is c-Src, a nonreceptor tyrosine kinase that controls proliferation, cell adhesion, and cell migration in normal and cancer cells. In this study we evaluated the functional and molecular effects of Src inhibitor PP2 in human ER-positive (MCF7 and T47D) and triple-negative (MDA-MB-231) breast cancer cell lines.

Consistent with our previous results, where we have demonstrated that Estradiol (E2)-induced cSrc activation is required for the disruption of Tight Junction proteins, in ER-positive breast cancer cell lines (Horm Cancer 5:161, 2014), here we show that E2 (1 nM) promotes $^{416}$Tyr-Src/$^{861}$Tyr-Fak (Focal Adhesion Kinase) complex formation, and increases *in vitro* cell migration (wound healing assay) and invasion (Transwell invasion assay). As expected, these effects were precluded when cells were incubated with the c-Src inhibitor PP2 (5 μM), in the presence of E2.

Surprisingly, when c-Src was suppressed by means of PP2 pre-incubation in the MDA-MB-231 cell line, the Src/FAK complex was formed, and *in vitro* cell migration and invasion increased, after 48 hours of E2 incubation, similar effects that were observed in the ER-positive cell lines stimulated with E2. These effects may be due to the restore of the ER expression or, perhaps, to the inhibition of its proteolysis.

Our study provides a new clue toward the understanding of the crosstalk between the ER and c-Src and represents a promising therapeutic strategy for TNBC cells that can be sensitized to antiestrogen-treatment.

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# GRP78 and ATF6 expression in low-high grade intraepithelial neoplasia and cervical cancer produced by HPV16.

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**Introduction.** The endoplasmic reticulum responds to different stress types through a sophisticated mechanism named "Unfolded Protein Response". This phenomenon consists in the activation of at least three molecular different branches, and each has a sensor and a transduction cascade. The main objective of this phenomena is the induction of genes that encode for chaperones, protein pleases, receptors or gene protein activators. In this way, this mechanism pretends to keep the cellular homeostasis, and in case of a persistent stress, the cell is conducted to apoptosis. The UPR has been involved with cancer development, this due to an induction o repletion failure seems to be related with the tumor survival. In this work we analyze the changes in the expression of GRP78 and ATF6 in precursor lesions and cervical cancer.

**Objective.** Analyze the changes in the expression of GRP78 and ATF6s in patients with low and high grade intraepithelial neoplasia and cervical cancer produced by HPV type 16.

**Methodology.** 56 women were selected previous informed consent, and were divided into 4 groups, 14 patients each; the group I women with hysterectomy for benign questions, group II women with low intraepithelial neoplasia HPV type 16, group III women with high intraepithelial neoplasia HPV type 16 and, group IV women with cervical cancer (adenocarcinoma, in situ, and epidermoid). All the samples were obtained from the Women Hospital of Aguascalientes and the General Hospital of Aguascalientes I, we obtained cervical swabs for the detection and typification of HPV. The patients with HPV type 16, were biopsied, the biopsy was divided in two pieces, one for immunohistochemistry and one for RT-PCR to analyze the genetic expression of GRP78, ATF6 and IRE1α.

**Results.**

We detected and typified HPV type 16 through multiplex nested PCR the samples. We choose HPV type 16 positive samples. Expression of GRP78 and ATF6 in the samples by immunohistochemistry were possible. The GRP78 is expressed in different degrees of cervical neoplasia. On negative controls the expression is punctual, basal and less than in intraepithelial neoplasia (low-high) and cancer. In Low intraepithelial neoplasia the signal is located on the basal cell of the epithelia, in severe intraepithelial neoplasia is in the superficial cells not in the injury and in cancer the expression is not punctual, is diffuse and close to blood vessels. The ATF6 expression in negative controls is basal, punctual similar to GRP78. In low intraepithelial neoplasia the signal is in the basal cells of epithelia, but is not only cytoplasmic is also nuclear, this means that ATF6 is activated.

**Conclusion.** GRP78 is possible a therapeutic target for the progression of the HPV infection. ATF6 is expressed in the intraepithelial neoplasia as well as cancer, less is known about the role of this protein in tumor survival.

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# Does *Fagopyrum esculentum* encode an ABC transporter involved in Aluminum tolerance?

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Aluminum (Al) toxicity is the main limiting factor crop production on acid soils. While most plants are sensitive to Al some species have developed strategies to cope with Al toxicity. These strategies are classified as exclusion and tolerance mechanisms. In the exclusion mechanism plants release organic acid anions (malate, citrate or oxalate) to sequestrate Al and prevent its uptake. In tolerance mechanism Al forms nontoxic complex with organic acids anions, and these are sequestered in root cell vacuoles, among such mechanism are those that quickly repair the damage caused by Al. In this mechanism there have been identified genes encoding proteins that form a complex and function as ABC transporters. ABC transporters represent a large family, they have two transmembrane domain (TMD) and two cytosolic nucleotide-binding domains (NBD). In rice, an ABC complex encoded by genes *OsSTAR1*/*OsSTAR2* participate in cell wall modification; in *Arabidopsis thaliana* the orthologue of OsSTAR2, *AtALS3,* is implicated in sequestrate Al in the root cell vacuole.

*Fagopyrum esculentum* (Polygonaceae), also known as buckwheat, is an important economic crop; this plant shows high Al resistance combining Al exclusion and Al tolerance mechanisms and can accumulate Al in their leaves. In a previous work a partial sequence of a gene of buckwheat was isolated; this partial sequence was named *FeALS3* because it shows high identity with *AtALS3*. Now we have isolated the complete cDNA sequence of *FeALS3*, this gene encodes a protein with hydrophobic regions suggesting that it might be a TMD protein. *FeALS3* contains only a TMD without NBD implying that it requires another protein that may form a complex with it. In Arabidopsis and rice genes *AtSTAR1* and *OsSTAR1* encode NBD proteins, which are implicated in aluminum tolerance, it is possible that a homolog of *STAR1* is present in Fagopyrum. We characterized a homolog of *STAR1* that was named *FeSTAR1.* We are currently analyzing by qPCR the expression of these genes at different times of Al exposure. Preliminary results show that *FeALS3* was induced by aluminum while *FeSTAR1* was not.

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# Function of the peroxisome ubiquitination complex in the sexual development of the fungus *Podospora anserina*

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Peroxisomes are single membrane bound organelles present in most eukaryotic cells. Their function importantly depends on the matrix proteins that are synthesized in the cytosol and imported into the organelle by two known pathways. One of them is mediated by the receptor PEX5 that recognizes proteins with the Peroxisome Targeting Signal (PTS) 1, and the other by PEX7 and its coreceptor PEX20 that recognizes proteins with the PTS2. Once the receptors are bound to their cargo proteins they interact at the peroxisome membrane with the docking complex, which consists of PEX13, PEX14 and PEX14/17, and translocate their cargo into the peroxisome matrix by forming a channel in the membrane. The docking complex interacts with the RING-finger complex (composed by the E3 ubiquitin ligases PEX2, PEX10 and PEX12) via the matrix protein PEX8 to form the importomer. Cargo translocation depends on the export of the receptors back to the cytosol, which is mediated by the exportomer and allows further rounds of import. The first step of the recycling process consists on the monoubiquitination of the receptors and coreceptor by the E2 ubiquitin-conjugating enzyme PEX4, which is activated and docked to the organelle by the membrane protein PEX22, and requires the activity of the RING-finger complex. The second step is the receptor translocation back to the cytosol. This process is mediated by the dislocation complex, which consists of the AAA ATPases PEX1 and PEX6 anchored to the organelle by PEX26. In the filamentous fungus *Podospora anserina* the absence of PEX20, PEX13 or the RING-finger complex blocks the sexual development before karyogamy and produces sterility. This defect is not seen in the absence of PEX5 and PEX7, which suggests that PEX20 mediates a third import pathway that is essential for karyogamy. To further support this model here we studied the function of the components of the peroxisome ubiquitination complex PEX4 and PEX22. We created gene-deletion strains for *PEX22* and *PEX4* and we demonstrate that they actually code for peroxisome biogenesis factors, which are required for the peroxisomal import of PTS1 and PTS2 proteins, but not of peroxisome membrane proteins, and we found that the sexual development of *Δpex22* and *Δpex4* mutants is blocked at the same stage as for *Δpex20* mutants, which is consistent with the model of the third pathway mediated by PEX20. This research was supported by PAPIIT grant IA201815 from DGAPA, Universidad Nacional Autónoma de México.

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# Activation of protein kinase C promotes $\alpha_{1B}$-adrenergic receptor late-endosome trafficking through Rab-9 interaction

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The G protein-coupled receptors (GPCRs) transmit a variety of signals across the cell membrane and regulate cellular activity via the intermediary role of guanine nucleotide regulatory proteins (G proteins). Among these receptors, the $\alpha_{1B}$-Adrenergic ($\alpha_{1B}$-AR) receptor mediates the effects of catecholamines, like epinephrine and norepinephrine, thorugh coupling to Gq. The activation of the $\alpha_{1B}$-AR causes polyphosphoinositide hydrolysis catalyzed by phospholipase C (PLC), C, generating inositol trisphosphate and diacylglycerol, which together lead to calcium signaling and protein kinase C (PKC) activation. Subsequently, receptors are phosphorylated, triggering their desensitization and internalization, through association to scaffold proteins, such as β-arrestin, clathrin and other proteins, includings Rab GTPases.

The Rab family of GTPases controls endocytosis, vesicular trafficking, and endosomal fusion. Among their remarkable properties is the differential distribution of its members on the surface of various organelles. In the endocytic pathway, Rab 5 controls traffic from the plasma membrane to early endosomes, whereas Rab 9 regulate the traffic from late endosomes to lysosomes and recycling to the trans-Golgi.

Because the fine mechanism that regulates $\alpha_{1B}$-AR trafficking remains unclear, we investigated the relationship between PKC activation and Rab GTPases in receptor trafficking. To accomplish this, we assessed the interaction between the $\alpha_{1B}$-AR and the GTPase Rab9 using Förster resonance energy transfer (FRET) and biochemical approaches, using cells co-expressing $\alpha_{1B}$-AR tagged with the red fluorescent protein, DsRed, and Rab9 protein tagged with the green fluorescent protein, GFP. We found that pharmacological PKC activation mimics $\alpha_{1B}$-AR trafficking elicited by non-related agonist such as sphingosine 1 phosphate (S1P), i.e. a transient receptor localization in early endosomes followed by a sustained presence in late endosomes. We showed that $\alpha_{1B}$-AR interacts in vesicular structures with the late endosome marker, Rab9, upon PKC stimulation. This interaction is abrogated after PKC blockadge and results in receptor retention at the level of the plasma membrane. Similar effects were observed when overexpressing either an $\alpha_{1B}$-AR mutated at PKC phosphorylation sites (S396, 402A) or when using Rab9-GDP, a dominant negative mutant. Finally, Rab9 GDP expression does not affect calcium response, but abolishes receptor desensitization.

This research has been supported by Grants from DGAPA and CONACyT. DAH-S and MAA-M are students of Programa de Ciencias Bioquímicas and GC-R is student of Programa de Ciencias Biomédica. All of them are recipients of fellowships from CONACyT.

# Analysis of the expression and subcellular localization of Retinoblastoma mutants.

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The RB protein was the first bona fide tumor suppressor discovered more than 29 years ago. The Rb pathway has been found altered, somehow, in virtually every cancer type. This protein and its family members are components of a canonical pathway that mediates cellular responses to a variety of stress signals; the mainly characterized role is the control of the cell cycle. Rb binds to and controls the transcriptional activity of E2F family members, regulating their target genes, needed for the cell cycle progression. When Rb protein is hyper-phosphorylated by the cyclin-dependent kinase complexes (CDKs), it becomes inactive and it dissociates from the E2F transcription factor. Although there has been intensive research on the role of RB (wild type) on the cell cycle, less is known about the impact of the mutations found in retinoblastoma tumors. For this reason we aimed to generate constructs from several commonly found mutations in RB in order to study their possible functions on the cell cycle, via association with E2F's transcription factors, subcellular localization, and their expression at the protein and mRNA level.

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# Expression of the Receptor for Activated C Kinase 1 in the photosynthetic dinoflagellate *Symbiodinium* during light/dark and growth phases.

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*Symbiodinium* are photosynthetic dinoflagellate algae that establish a mutualistic symbiosis with at least five phyla of marine organisms (Porifera, Foraminifera, Platyhelminthes, Mollusca and Cnidaria). The interest in understanding their biological mechanisms of symbiosis with cnidarians such as corals, anemones or jellyfish, has increased in the last decades due to the rise in frequency and severity of coral bleaching events. However, despite of the ecological significance of coral reefs, the understanding of the mechanisms that lead to the symbiosis establishment, as well as to its disruption (which leads to the coral bleaching) is still far from complete. In addition, suitable models for the study of these relationships are necessary as corals are difficult to propagate under controlled laboratory conditions. Thus, in an effort to shed light into these molecular mechanisms of signal-transduction, we have focused on identifying the signaling pathways that regulate the life cycle and light/dark responses of *Symbiodinium microadriaticum*, which is the symbiotic partner of the jellyfish *Cassiopea xamachana*. Our approach was to characterize a protein that is a hub in several signaling pathways, such as RACK1 (Receptor for Activated C Kinase 1; SmicRACK1), and to identify its ligands and expression patterns. *SmicRACK1* expression analysis by qPCR showed a differential accumulation with a maximal expression at the 6th and 20th days during growth in culture. Furthermore, its accumulation pattern fluctuated along the light and dark phases. These data suggest roles in active growth and proliferation, as well as light/dark cycle regulation in *S. microadriaticum*. We hypothesize that SmicRACK1 could have an active role in the proliferation regulation and light/dark induced changes during autonomous *in vitro* and endosymbiotic growth.

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# Phosphorylation of maize ribosomal proteins is stimulated by ZmIGF during germination

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*Zea mays* insulin-like growth factor (ZmIGF) stimulates germination of maize seed and seedling growth in a selective manner similarly as shown to occur with insulin. One of the most studied effects of insulin or insulin-like growth factors (IGFs) is the regulation of protein synthesis by activation of translational apparatus components through the PI3K-TOR pathway, including the translation of specific mRNAs particularly those coding for ribosomal proteins, such as rpS6 and translation factors or eEF1α. This peptide (ZmIGF) is known to be present in eukaryotes and it has been demonstrated that ZmIGF-stimulation targets tissues that induce phosphorylation of rpS6. These changes play an important role in post-translational control during the synthesis of ribosomal proteins.

Eukaryotic protein translation is mainly controlled at the level of initiation, which involves several events such as protein phosphorylation. Ribosome heterogeneity has been documented in different organisms, in which ribosomes change depending on the stage of development. These differences include qualitative and quantitative differences in ribosomal proteins and phosphorylation changes. It has been identified specific sites of post-translational modification by phosphorylation of the ribosomal proteins rpS2, rpS6, rpL13, rpL29, rpP0, rpP1, rpP2 and rpP3 in *Arabidopsis*.

The present research was undertaken to determine the phosphorylation changes that occur in the ribosomal proteins during germination of maize seeds with or without the addition of ZmIGF. To achieve this aim, maize seeds were germinated with or without ZmIGF and ribosomal proteins were isolated from embryonic axes. Phosphorylated ribosomal proteins were analyzed by SDS-PAGE 1D and 2D to determine differences in the patterns of phosphorylation of ribosomal proteins.

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# Leptin induces epithelial-mesenchymal transition in a Src-dependent pathway in mammary epithelial cells

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**Introduction:** Several experimental and epidemiological studies have linked obesity with the development and progression of breast cancer. Leptin is a hormone secreted by adipocytes that during obesity increases its production and experimentally has been shown that is involved in the development and progression of breast cancer through of various biological processes. Epithelial-mesenchymal transition (EMT) is a biological process of transdifferentiation where epithelial cells undergo morphological changes to a fibroblast-like shape acquiring an increase in cell migration and invasion. The loss of E-cadherin, gain of vimentin and the increase of cell migration have been considered canonical markers of EMT. Experimental studies have described that leptin promotes the EMT in breast cancer cells and in the non-tumorigenic epithelial cell line MCF10A, however, it has not been reported the role of Src kinase in the regulation of EMT canonical markers induced by leptin in mammary epithelial cells. **Methods:** Non-tumorigenic mammary epithelial cell line MCF10A was used in this study. Time course assays were performed and cell cultures were stimulated with leptin 400 ng/ml and Src activation was determined by Western blot. The role of Src on E-cadherin and vimentin expression as well as the increase of cell migration were evaluated by using the chemical inhibitor of Src, PP2. **Results:** The time course assays show that leptin induces an increase in Src activation at 5, 30 and 60 minutes. In addition, leptin promotes the increase of vimentin, N-cadherin, secretion of MMP-2 and MMP-9 as well as capacity of cell migration in MCF10A cells and these events are dependent of Src kinase activity. **Conclusion:** Leptin induces Src activation and regulates EMT markers through a Src-dependent pathway in mammary epithelial cells.

**Keywords**: Leptin, EMT, Src.

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# Importance of PKCθ activity in the balance of activation of transcription factors AP-1, NFAT and NF-kB in human CD4 T lymphocytes and its implication on neonatal immunity.

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The activation of T cells requires two signals, the specific signal mediated by the antigen receptor (TCR) and a signal of immunological alarm, which may be given by surface molecules or soluble molecules (cytokines).
In T cells, the TCR is responsible for recognizing the antigen peptide being presented by the MHC (major histocompatibility complex). Adequate signaling leads to activation of cells by inducing three main transcription factors:nuclear factor NF-κB, activator protein 1 (AP-1) and nuclear factor of activated T cells (NFAT). These three factors promote the expression of molecules that are crucial for cell function and cell activation,for which a proper balance of the three transcription factors is required.
It has been reported that cord blood (CB)CD4-T cells, compared with adult peripheral blood (APB) CD4-T cells, have a low production of signaturecytokines, particularly the Th1 cytokine IFNγ. Also, antigen presenting cells of newborns produce less IL-12, which is also required for Th1 differentiation.
The difference in the activation of transcription factors in adult and neonatal could be responsible for the low cytokine production in neonatal cells.A candidate molecule in the TCR signaling pathway that could be responsible for these differences is PKCθ, because it is responsible for the activation of AP-1 and NF-κB. Alternative pathways that could re-establish the balance between the main transcription factors may be important to achieve full activation of neonatal CD4-T cells. It has been reported that TLR5 signals are important co-stimulatory signals that promote cell proliferation and production of IFNγ, but the level of activation of transcription factors was not explored.
In this project, PKCθ activation is evaluated as well as its effect on the balance of AP-1 and NF-κB activation. Also, the effect of TLR5 signals is explored. We present our advances in the neonate and adult naïve CD4-T cell responses to the signals of TCR alone, TCR + TLR5 or TCR + CD28 on PKCθ and transcription factors activation.

# Effect of 100 IU of IL-2 on the proliferation of cervical carcinoma line HeLa and INBL

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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. The IFN-γ, IL-2, IL-12 and GM-CSF are excellent candidates as activators of the antitumor immune response and they have been used in various preclinical models with high effectiveness.

It´s well known that the cervical cancer cells lines HeLa and INBL has a functional receptor for IL-2, so this can be considered a good biological model to elucidate the effect of this interleukin in cervical cancer.

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 and the contrary high concentrations induce inhibition of proliferation. Therefore, we decided to treat cervical cancer cell with 100 IU of IL-2 to determine its effect on the proliferation on the different phases of the cell cycle of the cell line HeLa and INBL.

HeLa and INBL cells were treated with 100 IU/IL-2 for 48 and 96 hours and the proliferation was measured using crystal violet. For cell cycle we used the same times and PI incorporation was used to measure DNA content.

Fluorescence cells were analyzed by use of FACSAria II (BD) and flowing software software.

The treatment with IL-2, shown that the proliferation decreased with respect to the control and cell cycle was arrested in the G1-phase. Furthermore we observed that the treatment not induce senescence.

We concluded that the IL-2 is a candidate for adjuvant for treatment of cervical cancer.

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# GTPases Gpn1 and Gpn3 regulate nuclear entry of Rpb1, the largest subunit of RNA polymerase II, as a protein complex.

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Gpn1 and Gpn3, together with Gpn2, form the GPN-loop GTPase family, which received its name from a conserved structural motif formed by the peptide glycine-proline-asparagine. Gpn1 and Gpn3 are universally present in eukaryotic cells, which suggest they fulfill a fundamental cellular function. Consistent with this idea, deletion or silencing of any of these proteins in several species resulted in a loss of cell viability or embryonic lethality. Gpn1 and Gpn3 co-purify with Rpb1, the largest subunit of RNA polymerase II (RNAPII). RNAPII transcribes all protein-coding genes in eukaryotic cells and although is synthesized in the cytoplasm, it is functionally active in the cell nucleus. We previously demonstrated that Gpn3 is required for RNAPII activity by mediating the nuclear targeting of this enzyme (Calera *et al*., 2011, BBA-MCR. 1813:1708-1716).

In a recent study we demonstrated that Gpn1 and Gpn3 interact with each other, probably forming a stable functional heterodimer. This interaction is functionally important for both GTPases, as Gpn1 and Gpn3 protein levels drop until almost undetectable levels when the other protein is suppressed with a highly specific hsRNA (Méndez-Hernández *et al*., 2014, FEBS Lett. 588:3823-3829). Here we hypothesized that the strong interaction between Gpn1 and Gpn3 is relevant in regulating Rpb1 nuclear entry. To test this proposal we co-transfected HEK293 cells to express different versions of Gpn1 and Gpn3 proteins and examined the effect on the localization of Rpb1 assessed in immunofluorescence assays. Results will be discussed during the poster session.

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# Over-expression of Myelin and Lymphocyte Protein (MAL) reduces MUC1-C oncoprotein trafficking to the nucleus.

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MUC1 is a hetero-dimeric trans-membrane glycoprotein expressed on the apical surface of mucosal epithelial cells, but it is over-expressed and aberrantly distributed and glycosylated in transformed cells. Oncogenic activity of MUC1 relies on MUC1-carboxy-terminal subunit (MUC1-C), which is translocated to the nucleus, after extracellular MUC1-N subunit is released from the plasma membrane. In the nucleus MUC1-C 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. Several reports indicate that hyper-methylation of MAL gene significantly decreases MAL expression, and it is a feature found in different malignancies. On the other hand, transgenic expression of MAL protein, represses the formation of tumors in nude mice, decrease cell motility and induce apoptosis. We are interested to determine if the tumor suppressor activity of MAL coming from interfering with the MUC1-C oncogenic activity. So far, there is no experimental evidence published regarding MAL and MUC1-C relationship, however, it has been reported that MUC1 interacts with MAL-2, another member of the MAL family. The experimental approach included the generation of HEK293-cells stably expressing human MUC1 and MAL-GFP, transient transfections, and western blot analyses of subcellular fractions. We also tested protein interactions by immunoprecipitation and colocalization by confocal microscopy. Results show that MUC1 expression levels are comparable both in cells transfected with MAL-GFP and mock cells, but there are significant changes in MUC1-C levels in sub-cellular fractions. First, we found notable reduction (39%) of the amount of MUC1-C in nuclear extracts when MAL-GFP is expressed. Second, MUC1-C is also reduced (50%) in membrane fractions from MAL-GFP transfected cells, and third, seems that MUC1-C is accumulated in the cytoplasm of those cells (an increase of 58% more when compared with mock cells). We could not determine any MAL-MUC1-C interaction, then, we looked for changes in MUC1-C known associated proteins, as β-catenin. MAL apparently does not interfere with MUC1-C- β-catenin interplay. The next candidate is importin-β, which is the protein responsible for nuclear translocation of MUC1-C; we are working on it.

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# Extracellular vesicles from MDA-MB-231 breast cancer cells stimulated with linoleic acid promote migration through PI3K/Akt signaling pathway in MCF10A cells

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Breast cancer is the most common cancer and the main cause of cancer deaths in women worldwide. In Mexico, it is the first cause of death for malignant since 2006. Several studies suggest the association between a diet rich in fatty acids and the risk of breast cancer. Linoleic acid (LA) is an omega-6 polyunsaturated fatty acid (ω-6 PUFA) and the major PUFA in most diets. Recent studies indicated that the LA induces an epithelial-mesenchymal transition-like (EMT) process in MCF10A mammary non-tumorigenic epithelial cells and migration and invasion in MDA-MB-231 breast cancer cells. Extracellular vesicles (EVs) are membrane-limited vesicles secreted by normal and malignant cell. Exogenous stimuli including epinephrine, adenosine diphosphate, collagen, calcium ionophore (A23187) and amphiphiles induce MVs release in several cell types. Recent studies have shown that the EVs derived from MDA-MB-231 cells stimulated with LA promote migration in MCF10A cells. However the mechanism responsible for its effect is not well studied. Therefore in the current study, we evaluated the mechanism by which EVs induce migration in MCF10A cells. We demonstrate that EVs secreted by MDA-MB-231 cells stimulated with LA promote migration and activation of Akt2 in MCF10A cells. Moreover, we show that EVs from MDAMB-231 cells stimulated with LA promote migration through PI3K/Akt signaling pathway in MCF10A cells. EVs also promote activation of FAK and Erk1/2 in MCF10A cells. In summary, our findings demonstrate, for the first time, that EVs from MDAMB-231 cells stimulated with LA induce migration through PI3K/Akt2-dependent pathway in MCF10A cells.

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# Identification of the phosphorylated proteins by the Protein Kinase C during macrophage infection with *M. bovis*

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*Mycobacterium tuerculosis* (Mtb) is the causative agent of the Tuberculosis. Through time Mtb has developed strategies to manipulate the host's cellular machinery to impair key molecular mechanisms leading to its destruction, like phagosome-lysosome fusion. One of the known mechanisms involved in the phagosome-lysosome fusion blockade is the coating of the phagosome with Coronin-1 which leads to the induction of calcium fluxes that will in turn, activate the calcium-dependent phosphatase, Calcineurine. In line with the fact that the Protein Kinase C (PKC) family signaling pathway regulates basic processes of phagocytosis, it was recently shown that in INFγ activated macrophages, PKC through the phosphorylation of Coronin-1 inducing a switch from phagocytosis to macropinocytosis, leading to mycobacterium destruction. However, we have observed that during macrophages infection with *M. bovis*, PKC not only phosphorylates coronin-1 but also a large number of proteins with molecular weights ranging from 25 KDa to 250 KDa. Therefore, we propose that some of these PKC targets may be involved in the molecular mechanisms that prevent phagosome-lysosome fusion thus promoting macrophage infection and mycobacterium survival. To test this possibility, we are currently identifying the proteins phosphorylated by PKC during macrophage infection with *M. bovis* by a phosphoproteomic approach. The results of the phosphoproteomic analysis will be discussed.

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# Participation of ERK and Kpna2 in the nuclear translocation of Rac1 in HaCaT cells in response to estrogens

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**Introduction:** Rho GTPases play key roles during cancer development. Rac1 has a functional nuclear localization signal and it accumulates in the nucleus in different cell lines in culture. Nuclear-cytoplasmic shuttling of Rac1 is regulated by its interaction with the importin Kpna2 and the protein B23. Moreover, phosphorylation of Rac1 at Thr108 by ERK can regulate its nuclear import. Preliminary data from our laboratory shows that estrogen treatment induces nuclear accumulation of Rac1 in a non-tumorigenic keratinocyte cell line (HaCaT). The goal of this work was to determine if nuclear accumulation of Rac1 in response to estrogens in HaCaT cells, is mediated by Kpna2 and is dependent on ERK activation.

**Methods:** HaCaT cells were stimulated with 17β-estradiol (10μM). Subcellular localization of Rac1 was evaluated using immunocitochemistry and cell fractionation. Phosphorylation of ERK1/2 was determined by Western blot. Inhibition of ERK1/2 activation was performed using a chemical inhibitor and Kpna2 knockdown was performed using specific siRNA.

**Results:** Estrogen induces nuclear accumulation of Rac1 and phosphorylation of ERK1/2 in HaCaT cells. Inhibition of ERK1/2 activation or Kpna2 knockdown inhibits estrogen-induced nuclear accumulation of Rac1.

**Conclusions:** Nuclear accumulation of Rac1 in response to estrogens in HaCaT cells is dependent on Kpna2 and ERK1/2 activation.

**Key words:** Rac1, nuclear translocation, estrogens, ERK1/2, Kpna2.

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# A New MDM2-MDMX heterodimer independent of C-terminal RING-RING interaction induced by ATM.

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The p53 tumour suppressor induces the expression of a large number of gene products that have the capacity of control the cell cycle arrest, senescence DNA repair or apoptosis. The p53 degradation is regulated by MDM2, under normal conditions; MDM2 translocates the p53 protein out of the nucleus for degradation via the ubiquitin-dependent pathway. MDMX, a homologous of MDM2 does not harbor E3 ubiquitin ligase activity towards p53 and its negative activity is instead linked to suppression of p53 transactivity. Under genotoxic stress conditions, ATM phosphorylates both MDMX and MDM2 at Serines 403 and 395 respectively. These phosphorylation events stimulate *p53* mRNA translation and stabilization. Our results show that these phosphorylation events reside within intrinsically disordered domains and change the conformation of the proteins. The modifications promote the exposition of N-terminal interfaces that support the formation of a new MDMX:MDM2 heterodimer independent of the C-terminal RING-RING interaction. The E3 ubiquitin ligase activity of this complex towards p53 is prevented by the p53 mRNA interaction but, interestingly, does not affect the capacity to ubiquitinate MDMX and MDM2.

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# Characterization of a novel family of proteins involved in cholesterol transport.

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Cholesterol is an essential compound in mammalian cells because it is involved in a wide range of functions including an essential component of membranes, precursor of important molecules such as hormones, bile acids or vitamin D and also as a signalling molecule. The cholesterol transport across the circulatory system is a well-known process in contrast to the intracellular cholesterol transport which is poorly understood. Recently in our laboratory we identified a novel protein in *C. elegans* involved in cholesterol uptake, which we have named ChUP-1. In sillico analysis identified two putative orthologue candidate proteins in mammals. The proteins SIDT1 and SIDT2 share identity and conserved cholesterol binding (CRAC) domains with *C. elegans* ChUP-1. Both mammalian proteins are annotated as RNA transporters in databases. In the present study we show evidence indicating that SIDT1 and SIDT2 not only do not transport RNA, but they are involved in cholesterol transport. Furthermore we show that single point mutations directed to disrupt the CRAC domains of both proteins prevent FRET between SIDT1 and SIDT2 and the cholesterol analogue dehydroergosterol (DHE).

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# Role of TOR signaling pathway during *Azospirillum-Arabidopsis* interaction

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*Key words: Azospirillum, Arabidopsis, plant-microorganism interaction, target of rapamicyn*

The genus *Azospirillum* has the capacity to improve plant growth. The mechanism used by these bacteria to promoting plant growth is based in the production of phytohormones, leading to the modification of the architecture of the root system. However, aspects biochemical and molecular during the interaction are unknown. TOR (target of rapamicyn) is a protein related to the family of phosphatidylinositol 3-kinase-related kinases, initially identified in yeast. TOR protein is part of a signaling network that regulates cell growth and proliferation. The deregulation of the activity of this protein in animals, leads to the generation of diseases such as cancer, diabetes and others. The mutation of the gene encoding the TOR protein is lethal to plants, thus pointing to its role in plant development. Several studies have demonstrated the importance of this protein in regulating the development of the root system. Mutant plants with RNAi or treated with inhibitors have a lower development and growth. We decided to analyze the role of this protein in modifying the root system of *Arabidopsis* induced by *Azospirillum*. *Arabidopsis thaliana* (Col 0) seedlings were inoculated with different concentrations of *Azospirillum brasilense* Sp245. The inoculation reduced root length and induces lateral root formation, root hairs and increase in fresh weight. In addition, *tor-es* plants were treated with different concentrations of estradiol to inhibit TOR protein. Estradiol reduced root length at 0.1 µM. Studies are in progress to study the effect of *Azospirillum* on TOR pathway in *Arabidopsis.*

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# *EXPANSIN* gene family in *Lotus japonicus*: Identification, characterisation and expression analysis in response to symbiosis

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## ABSTRACT

Expansins constitute a diverse gene family in plants *viz.,* α-expansin (EXPA), β-expansin (EXPB), expansin-like A (EXLA), and expansin-like B (EXLB). Expansins are key players in cell wall expansion, growth and development. Though several research has been carried out in different directions, systematic and comprehensive study has not been focused on characterization of expansin gene family in *L. japonicus* and more particularly their expression pattern in roots followed by rhizobial inoculation or during nodule development. Herein, a total of 27 expansins were identified from *L. japonicus* genome using homology search and protein domain analysis. Further based on phylogenetic analysis we classified the expansin genes in to four subfamilies. The gene structure analysis using coding DNA sequences and genomic DNA sequences reveals most of the members within each subfamily share common exon-intron organisation. The divergence among few genes within the subfamily may arise in due course of evolution. Interestingly it was noticed that, members within the same subfamily shared the similar motif composition, order and type confirming their functional similarities. Motif 5 and 6 composed of 15 amino acids found to be highly conserved among all the expansins. Theoretical isoelectric point among all the expansins were ranged from 4.50 to 9.61 and the average molecular weight was found to be 27.04 kDa. The presence of signal peptide and cleavage site prediction by signal P 4.0 reveals that all the expansins contain signal peptides of 19 to 29 amino acid length except three. The expression analysis of expansin gene for tissue specific gene expression revealed differential expression pattern, 2 of 27 genes were upregulated whereas 9 were found to be downregulated in nodules. The impact and validation of these genes in regulating the transcriptional program in development of root nodules at different stages of rhizobial symbiosis is under progress. This work is supported by CONACYT-240614, PAPIIT (DGAPA-UNAM) grant no. IN219916 to M.L. and DGAPA-UNAM postdoctoral fellowship (DGAP/DG0639/2016) to S.K.M.

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# Effect of biotin on adipocyte differentiation

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Biotin is a water-soluble vitamin whose classical role is to act as carboxylases prosthetic group. It is now well documented that independently of its classical enzyme catalysis reactions biotin modify gene expression and participate in several biological functions such as development, glucose and lipid metabolism. Evidence exist that biotin is required for adipogenesis, however, the mechanisms that participate in this process are largely unknown. We evaluated the effect of biotin on the expression of adipogenic mRNA and proteins during adipogenesis and on steady mature adipocytes in the 3T3-L1 cell line.

The experimental design consisted in three groups: Sufficient (medium with Fetal Bovine Serum), Deficient (medium with Dialyzed Fetal Bovine Serum) and Supplement (medium with Dialyzed Fetal Bovine Serum and 1µM of biotin) conditions. The 3T3-L1 cells were seeded in culture dishes to attain confluence then, adipogenesis was induced 2 days after confluence with IBMX/Dexamethasone/ Insulin/10% of fetal bovine serum (i.e. Normal, dialyzed or biotin supplemented) for 48 hours. The culture medium was replaced with medium containing 10% of the respective serum and insulin. After 2, 3, 5 and 8 days, cells were harvested and evaluated for mRNA levels of *Pparγ, C/ebpα* and *Srebp-1c, adiponectin, holocarboxylase sintethase*. Adipose differentiation was evaluated as triglyceride content with Oil Red Oil staining.

We found that, irrespectively of biotin conditions, morphological differentiation was achieved in all groups, but the triglyceride content was lower in deficient treatment. Compared to the biotin-sufficient group, we found that during early adipogenesis (2-5 days) the mRNA levels of adipogenic genes: *Pparγ, C/ebpα, Srebp-1c* were not different between the groups. However, in mature adipocytes we found that biotin-deficient conditions decreased the mRNA levels of these factors as well as the mRNA expression of adiponectin. On the contrary, biotin-supplementation showed increased expression of these genes. We also evaluated the biotinylation level of carboxylases and the expression of holocarboxylase synthetase. Biotinylation of all biotin-dependent carboxylases were decreased in biotin-deficient conditions, and this effect was paralleled with mRNA levels of holocarboxylase synthetase. Surprisingly, since pharmacological effects of biotin have been reported to be achieved independently of carboxylase biotinylation, our data revealed augmented biotinylation level of all carboxylases

In conclusion, our data indicate that biotin does not participate in adipogenesis, but is required to maintain the expression of lipogenic genes in the mature adipocyte. In pharmacological conditions, biotin increase the mRNA levels of lipogenic factors in mature adipocytes without changes on triglyceride content, and also increase biotinylated-related proteins.

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# The expression of NR4A receptors and autophagy as part of the DNA damage response

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Genome integrity is a crucial factor to preserve cell viability, then cells must be able to detect and in case repair genomic lesions generated as consequence of the exposure to endogenous or exogenous agents. Thereby the accumulation of DNA damage underlay cellular processes like physiological aging or pathological cancer development. The cellular response to these genomic threats include the activation of molecular machineries to deal with different lesions, besides, as part of the DNA damage response, it has been reported the induction of autophagy which could imply opposite results, cell survival or cell death, depending on the resolution of DNA insults.

Another consequence of the genomic threat is the recruitment of orphan nuclear receptors NR4A to DNA damage foci, so the NR4A1/NR42 downregulation implies DNA repair defects. On the other hand NR4A1 has been described as a necessary element for autophagic cell death, implying a possible involvement of NR4A1 as a central modulator of macroautophagy. This arise the question if DNA damage response and autophagy are simultaneously modulated by NR4A and if this includes only a gene expression control or influencing protein-protein interactions.

To prove this, we have stablished a DNA damage and repair model using mouse embryonic fibroblast (MEF) and A549 tumor cell line exposed to Etoposide which results in DNA double strand breaks. We have detected an early induction of autophagy during DNA damage and along the repair process. Interestingly, we have observed that an induction of autophagy previously to DNA damage protects DNA from double strand breaks in MEF but in tumor cell line, it has the opposite effect. Simultaneously we observed a localization of NR4A1 / NR4A3 but not NR42 at the nucleus as the DNA damage marker γH2AX.

Even more our results highlight the relevance of autophagy in genome integrity, and show that nuclear orphan receptors NR4A1 /NR4A3 could be modulating DNA damage response including autophagy.

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# Role of Ser/Thr or Tyr protein kinase phosphatases on MAPK Tmk3 signaling in *Trichoderma atroviride*

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The intracellular signaling pathways are vital mechanisms by which organisms transduce environmental cues to locate their environment, respond appropriately and adapt to the conditions prevailing in their environment. Light is an abiotic environmental factor that regulates the behavior of virtually all living forms, such as conidia production, growth, reproduction, metabolism and circadian rhythms. The main system of light perception in fungi are the BLR proteins (Blue Light Regulator), homolog to WC1/2 photoreceptor complex of *Neurospora crassa*, however, there are evidence that light is transduced by different signaling pathways conserved in eukaryote. In *T. atroviride*, light induces asexual reproduction and gene regulation by activating of MAPK (Mitogen-Activated Protein Kinase) Tmk3 signaling that integrate stress and light signals (Esquivel-Naranjo *et al*., 2016). The activation by phosphorylation is transient in Tmk3, suggesting that a mechanism of response could consist of specific phosphatases for each kind of stress. The MAPK Tmk3, homologous to Hog1 of *Saccharomyces cerevisiae*, is involved in responses to different kind of stress, conidia production and expression of genes regulated by light (Esquivel-Naranjo *et al*., 2016).
Phosphatases with activity on Ser/Thr or Tyr protein kinase were identified by *in silico* analysis using as reference the phosphatases Ptc1, Ptc2 and Ptc4 that regulate the phosphorylation of Hog1 in *S. cerevisiae* (Warmka *et al*., 2001), and Pyp1 and Pyp2 tyrosine phosphatases, which control the phosphorylation status of Sty1 in *Schizosaccharomyces pombe* (Kowalczyk *et al*., 2013). To determine the role of phosphatases and the relationship with Tmk3 in stress responses, mutant strains lacking the coding regions of the phosphatases will be obtain using the double-joint PCR technique and the *hph* gene conferring hygromycin resistance in fungi. As a second approach, the phosphatases will be overexpress using the constitutive expression plasmid pUE08.

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# Overexpression of nodulin 41 in transgenic roots of *Phaseolus vulgaris* and identification of its putative substrate

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In nitrogen-limited conditions, many legumes enable symbiotic associations with soil bacteria named rhizobia, which can convert atmospheric $N_2$ to molecular forms that are incorporated into the plant metabolism. This interaction involves a complex molecular signaling between both partners in order to develop new organs, the root nodules, where the symbiotic nitrogen fixation occurs. Along the nodulation process many plant genes are specifically activated, known as nodulin genes, and depending on the timing when they are expressed they can be considered early or late nodulin genes. Gene products of the former group are mainly involved in early chemical signaling and nodule initiation and/or development, whereas products of the latter group are involved in nodule metabolism. We are interested in the study of nodulin 41 (PvNod41), a late nodulin of *Phaseolus vulgaris* (common bean) which is highly accumulated after day 14 during the nodule development. By a biochemical approach we have been able to purify this protein, and found that is an atypical aspartyl peptidase that has the ability to bind to denatured proteins, but with a restricted peptidase activity against diverse protein substrates. PvNod41 is similar to *Arabidopsis* CDR1, an extracellular atypical aspartyl peptidase that cleaves an unknown substrate and generates a systemic signal involved in pathogen resistance. Since the identity of the putative substrate or substrates of nodulin 41 are yet unknown, a cassette coding for PvNod41-6XHis protein was overexpressed in transgenic roots of *P. vulgaris* to identify interacting proteins by co-purification in immobilized-metal affinity chromatography (IMAC). At least three different proteins co-purified with PvNod41-6XHis after IMAC of a protein extract from transgenic roots. Currently, these proteins are in process of identification by LC-MS/MS and *de novo* peptide sequencing. Undoubtedly, this information will be relevant to establish the subcellular localization and biological function of PvNod41.

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# Identification of proteins related to the signal transduction in syncytiotrophoblast mitochondria

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Protein phosphorylation in the syncytiotrophoblast mitochondria (SM) is mainly supported by the PKA kinase activity, whose inhibition results in a decrease of progesterone (P4) synthesis and, particularly, in an increase of phosphorylation in some proteins. Nevertheless, SM contains several proteins phosphorylated in Ser, Thr and Tyr amino acids, suggesting a broad activity of kinases and phosphatases, as well as proteins associated with phosphorylation/dephosphorylation system. To determine the composition of this pathway, we used two study models: the JEG-3 cells and purified mitochondria, and submitochondrial fractions from syncytiotrophoblast.

Previous reports indicate the existence of membrane and soluble isoforms of adenylate cyclase proteins (AC) in syncytium cells. In this work, mAC3, mAC9 and sAC isoforms were identified in submitochondrial fractions using antibodies. Also, a Ser/Thr mitochondrial protein phosphatase 2C (PP2C family) was identified in SM. Recently, it has been reported that PP2Cm (PP2C mitochondrial isoform) regulates the opening of mitochondrial membrane permeability transition pore (MPTP), which is essential for cell survival. However, when JEG3 cells incubated with sodium fluoride, inhibitor of PP2C, the P4 synthesis was repressed and cells viability was maintained. Therefore, a different mitochondrial PP2C isoform can be activated/inactivated during the cellular signaling events associated with steroidogenesis.

It is known that different phosphodiesterases (PDE) can regulate different cAMP pools in diverse biological processes. PDE 2A, 4, 5, 8A and 8B isoforms have been characterized in steroidogenic tissues; particularly, PDE3 and PDE4 isoforms were identified in placental explant culture as a major regulatory mechanism associated with autocrine/paracrine signaling effects mediated by cAMP-PKA activation. Thus, the PDE activity in the P4 synthesis was evaluated in JEG-3 cells, where the inhibition of phosphodiesterase with olprinon resulted in a decrease of P4 synthesis.

The inhibition of steroidogenesis by the inactivation of regulatory proteins in signaling pathways, suggests that there is a relationship between these processes. In order to identify the proteins involved in the phosphorylation/dephosphorylation process, membrane fractions of SM were analyzed by mass spectrometry. Also, phosphorylated peptides purified from mitochondria of JEG-3 were analyzed in the same way. The results will let us identify proteins of cell signaling which modulate steroidogenesis and further establish a possible mechanism of signal transduction.

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# Activation of RhoGTPases, Rac and Cdc42, by calcium-sensing-receptor mutants found in breast cancer patients.

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The calcium-sensing-receptor (CaSR) belongs to the family class C of GPCRs, which can regulate cell proliferation, calcium homeostasis and secretion, among other processes, depending on the tissue in which this receptor is expressed. Its physiological importance is also evidenced by several disorders in the calcium homeostasis associated with a number of naturally mutations in the CaSR sequence. The bioinformatic study of the CaSR in the catalogue of somatic mutations in cancer (COSMIC) has documented 415 mutations of this receptor. Fourteen of these are specifically found in breast cancer patients, four of them ($CaSR_{N639K}$, $CaSR_{T732A}$, $CaSR_{R886Q}$ and $CaSR_{V894I}$) were generated and study previously in our laboratory to analyzed their role in the secretion of chemotactic factors. Importantly, Rho GTPases, molecular switches which regulates actin cytoskeleton, have been involved in the secretion of chemotactic factors. In order to investigate whether CaSR mutations might regulate secretion through the activation of Rho-GTPases, we first assessed the activation of Rac and Cdc42 Rho-GTPases in HEK-293 cells transiently transfected with CaSR Wild type ($CaSR_{Wt}$), $CaSR_{N639K}$, $CaSR_{T732A}$, $CaSR_{R886Q}$ or $CaSR_{V894I}$ mutants by using pull-down assays to capture the active form of Rac and Cdc42. Our results demonstrated that stimulation of $CaSR_{Wt}$ promoted Rac activation, reaching a maximum level of activation of this GTPase at 3 minutes of stimulation. However, for Cdc42 the maximum level of activation was reached after 10 minutes of $CaSR_{Wt}$ stimulation. These results suggest that CaSR might promote the activation of different Guanine Exchange Factors, which can in turn active Rac and Cdc42, respectively. Likewise, we compared the ability of the CaSR mutants on Rac and Cdc42 activation, we found that $CaSR_{N639K}$, $CaSR_{T732A}$, $CaSR_{R886Q}$ and $CaSR_{V894I}$ mutants presented different patterns on the activation of these GTPases compared to $CaSR_{Wt}$ in both basal and stimulus conditions. Moreover, in order to understand whether these differences were due to changes in the signal transduction promoted by these mutants, we examined the effect of PTX (pertussis toxin), an inhibitor of Gi protein; H89, a PKA inhibitor; and U73122, a phospholipase C inhibitor on ERK activation. We found that $CaSR_{Wt}$ as well as the $CaSR_{N639K}$, $CaSR_{T732A}$, $CaSR_{R886Q}$ mutants were able to activate ERK through Gi. Meanwhile, $CaSR_{V894I}$ as well as $CaSR_{R886Q}$ mutant were sensible to the effect of H89, which means that these mutants are able to signal through Gs protein. Surprisingly, the change in the amino acid R886Q on CaSR sequence promoted that this mutant have an apparent affinity for both Gi and Gs proteins. Altogether, these results demonstrate that CaSR mutations have difference in the G-protein coupling which might have impact on Rac and Cdc42 activation.

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# Role of β-arrestin2 in signaling and regulation of Corticotropin-Releasing Factor Receptor Type 1 ($CRF_1$).

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CRF (Corticotropin-Releasing Factor) is a neuropeptide released as a neuroendocrine response to stress. It acts through its receptors, $CRF_1$ and $CRF_2$, members of the G protein-coupled receptors (GPCR) family. $CRF_1$ receptors are mainly coupled to Gs- and Gq- proteins, which in turn activate PKA and PKC, respectively. Moreover, it has been reported that $CRF_1$ receptors can activate the mitogen-activated protein kinase (MAPK) pathway through transactivation of EGF receptor. β-arrestin2 is an adapter protein that participates in the regulation of GPCRs and has important functions in cell signaling mediating activation of different transductional pathways, including MAPK pathway. In the present work, we used mouse embryonic fibroblast from knockout mice that lack β-arrestin2 (MEF KO-β-arrestin2) transfected with HA-h$CRF_1$ receptor (MEF KO-β-arrestin2-HA-$CRF_1$) to elucidate the role of β-arrestin2 in the activation of MAPK induced by CRF. We found that the absence of β-arrestin2 promote a desensitization state that is reflected as an increase in the levels of cAMP produced in response to CRF. Our data also revealed that CRF stimulates ERK1/2 activation through β-arrestin2, mechanism that also depends of Src activation and transactivation of EGFR. This work was supported by CONACYT grant 167673 to JAOR and CONACYT scholarship 296029 to GKPM.

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# The Human Papillomavirus type 16 E5 and Ras Oncoproteins control cell differentiation to favor transformation.

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**Introduction:** The E5 oncorpotein from HPV16 (HPV16-E5) modulates the keratinocyte differentiation, which is important to allow the progression of viral cycle for generation of viral particles, or cellular transformation in persistent infections when HPV16 ***E6*** and ***E7*** oncogenes become over-express. Also, the E5 oncoprotein synergized with the EGFR to control the cell cycle through the modulation of $p27^{Kip1}$ levels. In addition, the Ras protein is part of the EGFR signaling pathway and together with E5 are regulating cell division and differentiation.
**Objective.** To characterize the molecular mechanism through which HPV16-E5 and Ras modulate cell differentiation to allow progression of viral cycle, or cellular transformation.
**Methods.** Cell lines derived from human (HaCat) and mouse (NIH-3T3) origins were transfected with HPV16-***E5***, alone or combined with ***Ha-ras*** oncogene. Cell transformation was measured by growth curves, FACS cell cycle analysis and colony formation in soft agar, in the presence or absence of EGF. To evaluate the degree of differentiation, normal and E5-expressing cells were grown in 0.2% FBS and high $CaCl_2$ concentration for different periods of time. Levels of Cytokeratin 14 (CK-14, undifferentiated cells) and CK-1 (differentiated keratinocytes) and other differentiation protein markers were evaluated by Western blot.
**Results.** We observed a non-additive activity between Ras and E5 in cells expressing both oncogenes, as a reduction in proliferation and transformation was detected. On the other hand, when cell differentiation was evaluated by the cytokeratins pattern, it was observed that E5-expressing cells presented low levels of cytokeratins as compared to HaCat control cells. When cells were induced to differentiation with calcium, the CK-14 was reduced in HaCat cells, but it was absent in E5-expressing cells as well as the CK-1. Experiments are in progression to evaluate the role of Ras and E5 together during keratinocytes differentiation.
**Conclusions.** The activities of HPV16-E5 and Ras are not additive during cell transformation process as it is observed with HPV16-E7 and Ras. The null or low levels of cytokeratins in E5-expressing cells suggests that the differentiation process has been arrested to maintain the viral cycle in the cells, but if this process persist the cell could loss control and become transform.

# TLR and MyD88 identification in the crayfish *Cherax quadricarinatus*.

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The molecular recognition of non-self antigens is a key in the immune innate response, however, is not easy because the molecular heterogeneity in pathogenic molecules, the major strategy is the recognition of pathogen-associated molecular patterns (PAMPs) by receptors that identify conserved non-self molecules. In *Drosophila*, pathogen recognition by Toll receptors is central to the activation of innate immune responses; TLRs can interact with distinct PAMPs derived from bacteria and fungi. . In crustaceans are few reports of molecular identification of TLRs. The purpose of this research was to identify two components of the TLR signaling pathway (TLR-4 and MyD88). We identified in *C. quadricarinatus* two proteins from hemocityc lysates by using anti-TLR4 antibodies in chemiluminiscent wester blot substrates with molecular weight of 20.7 and 139.2 kDa; this data is the first report, in crustaceans for a TLR4 with those molecular weight, the TLRs previously identified, have a molecular weight between 106 (*Fanneropenaeus chinensis, Litopenaeus vannamei* and *Penaeus monodon*) and 115 kDa (*Procambarus clarkii*), as predicted from the gene sequence reports; and also is the first report with a small molecular weight for a TLR4, perhaps, this could be the result of a enzymatic cleavage. The MyD88 antibody recognizes a protein of 70 kDa, the previous reports for this protein are from gene sequence reports and are around 53 kDa in peneid species (*P. monodon, F. chinensis and L. vannamei*). Also we identify the cellular position of both proteins, the TLR4 are in the plasmatic membrane and the MyD88 is located in the cytoplasm. In summary, this study of TLR-4/MyD88 identification in *C. quadricarinatus* supported the view that an evolutionarily conserved TLR signaling pathway may exist in shrimp as in the vertebrates and provide valuable information for the study of origin and diversification of immunity, in the future we can understand the innate immune pathway with the aim of prevent some diseases in crustacean's aquaculture.

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# Effect of tranilast and raloxifene treatment in the protein expression of e-cadherin in human breast cancer cells

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Breast cancer is the foremost cause of death of women with cancer in México. The main reason for the patient's death is the metastasis of cancer cells, which involves the expression of cell adhesion protein E-cadherin and some transcription factors such as Snail. The drugs used for treatment of this pathology have different effect depending on the subtype of breast cancer, for example if it depends on estrogen to proliferate, or whether it is invasive or not. Raloxifene is a drug of hormonal treatment widely used in estrogen receptor dependent breast cancer, which has proven effective in reducing tumor growth. Although, more studies on its effectiveness in cells without expression of this receptor are needed. Tranilast is an antiallergic drug that has demonstrated activity in reducing the proliferation of neoplastic cells, however, it is unknown whether it could decrease cell migration associated with the expression of adhesion proteins such as E-cadherin or its repressor Snail. Because of this, it is important to perform studies on the combinatorial effect of Raloxifene and Tranilast on migratory activity of breast cancer cells. These studies were conducted using the technique of cell culture of breast cancer, making a curve-concentration response for each drug in order to determine the concentrations to be evaluated. Finally, the effects on the expression of E-cadherin and Snail were analyzed by western blot and the changes in the cellular localization of the Snail protein were observed by immunocytochemistry.

# Role of $Ca2^{+}$ in the □$_1$-adrenergic receptor-mediated $H_2O_2$ synthesis.

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NADPH oxidase type 2 (Nox2) catalyzes the electron transport of cytosolic NADPH to molecular and generates $O_2^{\cdot -}$, which is converted to $H_2O_2$. Previous studies in the laboratory showed that adrenaline modulates Nox2 in liver cells: $\alpha_1$-adrenergic receptors (AR) activation increases $H_2O_2$ synthesis, and β-AR activation decreases $H_2O_2$ pool. Selective activation of $\alpha_1$ or β-AR stimulates the rates of gluconeogenesis, ureogenesis and glycogenolisis. Negative cross talk between $\alpha_1$-/ β-AR for $H_2O_2$ synthesis was observed. In addition, negative cross talk for $\alpha_1$-AR via $H_2O_2$ to β-AR-mediated stimulation was recorded in the mentioned hepatocyte metabolic routes. Furthermore, $H_2O_2$ impaired the β-AR-mediated stimulation in the same metabolic routes (Ki 0.1 μM). Present work expands such information: 1) $\alpha_1$-AR activation in hepatocytes requires the presence of calcium to generate $H_2O_2$, 2) this activation causes increased intracellular calcium, 3) the $H_2O_2$ increases intracellular calcium, 4) the calcium released by $\alpha_1$-AR activation comes from the pools that are in the cell, 5) β-AR activation in hepatocytes does not increase intracellular calcium. An integrated scheme with all the available information will be presented.

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# Paracrine Induction of Senescence by Mammalian Brain Cells *in vitro*

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Cellular senescence is a phenotype defined by a permanent cell cycle arrest, chages in gene expression and the secretion of cytokines, chemokines, growth, factors, proteases and reactive oxygen species, collectively known as Senescence Associated Secretory Phenotype (SASP). Other changes associated with cellular senescence include large, flattened and vacuolarized morphology, incresed lisosomal mass and elevated expression of cyclin dependent kinase inhibitors (p21/CDKN1A and p16/INK4A). Senescent cells can be distinguished from quiescent cells by their morphology, their lisosomal senescence associated beta galactosidase activity and incresed expression of p21/CDKN1A and/or p16/INK4A. Physiologically, senescent cells have a role as tumor suppressors, contribute to amniote embryonic development and optimal wound healing. However, they accumulate with age and contribute to the development of multiple diseases, mainly due to the effects of the SASP in non tissue remodeling contexts. Molecules secreted by senescent cells promote the establishment of both autocrine and paracrine senescence, tissue remodeling, inflammation and counterintuitively in cancer progression. In spite of the fact that neurons are postmitotic cells, they acquire many characteristics of senescent cells in aged organisms. We have developed an *in vitro* model of senescence of primary cultures of rat cortex. In this work, we investigate the potential paracrine induction of senescence by molecules secreted by senescent cortical cells over prenatal cortical neurons, astrocytes and mouse embryonic fibroblasts.

**Key words:** cortical neurons; aging; cellular senescence; SASP; mammals

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# Dynamics of the phospholipid flippase DNF-2 in *Neurospora crassa*

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Phospholipid membrane asymmetry is an important topography feature for vesicle formation during endocytosis. The production of a curvature in the plasma membrane seems to be a driving force for endocytic vesicles formation. The proteins involved in this phospholipids translocation to the cytosolic leaflet of the membrane are the flippases. These proteins are P-Type 4 ATPases that produce a shift in the charge and composition of the inner leaflet of the membrane. In this work we studied DNF-2 that is an aminophospholipid flippases responsible for the maintenance of the asymmetry of the membrane. We produced a *dnf*-2 gene deletion mutant and a strain expressing the chimeric protein DNF-2-GFP to assess its dynamic and organization in living cells of *N. crassa*. DNF-2-GFP was localized in the core of the Spitzenkörper, similar to the localization of the chitin synthases but it was completely absent in septa. Δ*dnf*-2 mutant had a growth rate reduction of 42.34% in comparison with the wild type strain. Conidiation was affected producing only 50.1% of the total conidia produced by wild type. Cells of the Δdnf-2 mutant, stained with the lipophilic dye FM4-64, showed a distorted hyphal morphology with periods of polarized growth intercalated with periods of isotropic growth. The Spitzenkörper (Spk) was unstable and it was divided in several growth sites. The division of the Spk produced apical branches constantly. These results suggest that DNF-2 is not essential but is involved in the Spk stability and cell growth.

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# TOR modulates AUXIN expression patters during root nodule symbiosis in common bean

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## ABSTRACT

Auxin is a key hormone in plant morphogenesis influencing cell division, elongation and differentiation. Polar auxin transport and gradient auxin distribution are necessary for correct distal pattern formation in plants, including early embryogenesis and root development. The most widely used tool to visualize auxin distribution is the synthetic DR5 based auxin inducible reporter. Using the promoter of DR5 reporter, auxin distribution and signaling responses have been well-documented in *Arabidopsis*. In *Medicago* the auxin distribution patters during the development of de novo organ, nodule was determined using DR5 reporter. Very recent report shows that the evolutionary conserved central growth regulator TARGET OF RAPAMICIN (TOR) acts as a key player of auxin signaling indicated the communication between TOR and auxin signaling exists in plants. With this background herein, we intended to determine the expression patterns of DR5 during root nodule development under TOR downregulated conditions. To achieve this we downregulated the TOR gene expression in the model grain legume *Phaseolus vulgaris* (common bean) roots that constitutively express DR5::GUS/GFP reporter. These transgenic roots were further inoculated with *Rhizobium etli* and a detailed high resolution microscopy imaging revealed the auxin behaviour during early cortical cell divisions, nodule primordia, young and mature nodule development. This work will also present the expression patterns of DR5 in senescence nodules. Together, our results show TOR regulated auxin expression patters during root nodule symbiosis in common bean. This work is supported by CONACYT-240614 to M.L and partially by PAPIIT (DGAPA-UNAM) grant no. IA205115 to MK.A.

# Dynamic network of Mdm2 and p53 interactions.

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The protein p53 is the main tumor suppressor and its main regulator is the protein Mdm2. Mdm2 is considered a hub protein due to its capacity to interact with a large numbers of different partners of which p53 is most well described. MDM2 is an E3 ubiquitin ligase, and many, but not all its interactions relate directly to this activity, either as substrates, adaptors or bridges, promoters, inhibitors or complementary factors. Additionally, Mdm2 is subjected to a multitude of post-translational modifications and is expressed in different isoforms that allow it to interact with more than 100 different partners. In this work we create a "Booleano" model. In this model each protein could be represented by two states; 1) active or 0) inactive. For p53 and Mdm2 we used three different states due to the where 2) represent phosphorylated form of the proteins. Booleans tables were created from experimental data recollected from 26 proteins belonging to different signaling pathways that interact with Mdm2, p53 or both. All initials conditions for each protein have been assay and the steady states are analyzed in order to observed a pattern of initial conditions associated to cancer. Likewise, to analyze the hub proteins able to lead to these patterns

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# Extracellular vesicles from women with breast cancer promotes migration in MDA-MB-231 breast cancer cells

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Breast cancer is the most common cancer and the leading cause of death in women worldwide and affects countries at all levels of development. Extracellular vesicles (EVs) are small membrane-enclosed sacs of endosomal and plasma membrane origin secreted by normal and malignant cells. The EVs represent an important mode of intercellular communication by serving as vehicles for transfer between cells of membrane and cytosolic proteins, lipids, and RNA. Particularly, EVs from tumor cells mediate many stages of tumor progression including angiogenesis, escape from immune surveillance, invasion and metastasis. The aim of this work was to determine the EVs number in plasma of breast cancer patients and healthy women, and whether EVs from breast cancer patients are able to induce migration of MDA-MB-231 breast cancer cells. We analyzed plasma fractions enriched in EVs and deprived of platelet-derived EVs obtained by differential centrifugation from blood samples of twenty-one Mexican female patients (median age 54 years, range 38-80 years) with biopsy-proven of breast cancer at different clinical stages and without receiving therapy. The control group consisted of 13 healthy females (median age 42.7 years, range 16-86 years). EVs number was evaluated by BD TruCOUNT Tubes (BD Biosciences) and migration was studied by scratch-wound assay. Our findings demonstrated that EVs number is higher in women with breast cancer with stages II and III compared with control group. In addition, EVs from breast cancer patients are able to induce migration of MDA-MB-231 cells and secretion of MMP-9.

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# Leptin regulates expression and subcellular localization of epithelial-mesenchymal transition markers in MCF10A human mammary epithelial cells

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**Introduction:** The epithelial-mesenchymal transition (EMT) is the process by which cells change from an epithelial to a mesenchymal phenotype, it is characterized by changes at molecular and cellular level, including a decrease or sucellular localization of proteins associated with cell-cell adhesions like E-cadherin, and increase of proteins like N-cadherin and vimentin. In addition, mesenchymal cells undergo reorganization of the actin cytoskeleton, loss of apico-basolateral organization and an increase of migration and invasion capacities; EMT is often associated with various human cancers because some markers of mesenchymal phenotype were observed during the progression of breast cancer. One of the main risk factors associated with the development and progression of breast cancer is the obesity which generates a rich microenvironment in hormones that can promote EMT; some reports indicate that leptin is one of the adipocytokines that promotes this process of cell transdifferentiation in various cell lines. **Objective**: To evaluate canonical EMT markers in MCF10A cells stimulated with leptin. **Methods:** Cell cultures MCF10A were treated with different dose of leptin, morphological changes and expression and subcellular localization of vimentin and E-cadherin proteins were determined by fluorescence and Western blot. **Results:** Leptin modifies the subcellular localization of E-cadherin, promotes an increase in vimentin expression and induces changes in cell morphology in MCF10A cell line. **Conclusion:** Leptin induces modifications in subcellular localization and molecular changes of proteins involved in epithelial-mesenchymal transition process in MCF10A cells.

**Keywords: Leptin, EMT, vimentin, E-cadherin**

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# Participation of the MPK6-MKP1 module in the responses of the *Arabidopsis thaliana* root to L-glutamate

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There are several biotic or abiotic factors that positively or negatively alter the plant root development; among the biotic factors are the microorganisms that can interact with the plant root by secreting several distinct bioactive molecules such as amino acids, peptides and phytohormones. It has been reported that the amino acid L-glutamate (L-Glu) modifies the *Arabidopsis thaliana* root architecture, repressing primary root growth and promoting lateral root development, effects similar to those cause by the phytohormone auxin [1]. The components of L-Glu signaling pathway involve the participation of the MEKK1 protein, which belongs to the group of Mitogen-Activated Protein Kinases (MAPKs) [2]. Previous studies from our group suggest the involvement of MPK6, a MAPK, and its negative regulator, the MKP1 phosphatase, to act on the signaling pathway of L-Glu. In this study, we demonstrate that L-Glu signaling targets the auxin pathway to modulate meristematic activity and root developmental programs. Besides, we evaluate the changes produced by the L-Glu in the levels of auxin transport proteins and regulators of the root stem cell niche, which suggest an important role of L-Glu in root architecture remodeling.

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# Role of UBEs AhR and RBX1 in the ubiquitin proteasome proteolytic pathway for Breast Cancer development

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The protein degradation process in eukaryotes is driven through the ubiquitin proteasome system (UPS). In humans, alterations in UPS are associated with hormone-dependent breast cancer (HBC) development, mainly treated with tamoxifen, an inhibitor of the alpha-estrogen receptor (ERα) that blocks the tumor progress; moreover, not all HBC patients respond to this treatment, but an independent alternative to prevent the union of estrogen to ERα is the degradation of this receptor. This degradation process is carried out by UPS, through the ubiquitinating enzymes (UBEs) AhR and RBX1 (UBEs type E2 and E3 respectively), that recognize this receptor. In the present work, the role of AhR and RBX1 in the degradation process of the ERα was evaluated in biopsies of women without HBC (controls) and with HBC (clinical cases). Samples were divided according to estrogen receptor (ER) levels, which represent the most abundant form, ERα. Subgroups are as follows: women not expressing ERα (ER-); women with ERα<50% (ERα<50%); and women with ERα>50% (ERα>50%). The participation of AhR and RBX1, was performed by RT-qPCR or immunohistochemistry, then it was determined if changes in protein levels of ER were due to UPS. In the ER- group, RBX1 showed an increment on its expression compared with ERα<50% and ERα>50% groups, which correspond with the expected results: at higher level of RBX1, lower are the ER levels, owing to a greater ubiquitination followed by degradation of the ERα. In the ERα<50% group, AhR participates as a transcriptional factor instead of as UBE. High expression levels of RBX1 are not sufficient to regulate ubiquitination and degradation of ERα; it is a requirement to have participation of both enzymes AhR and RBX1. The high expression of RBX1 and the potential enzymatic activity of AhR as UBE, are complement of each other to explain lower levels of ER in the ER- group respect to the ERα<50% group. Our findings strongly suggest that changes in ER levels are dependent of the UPS through the joint participation of UBEs AhR and RBX1.

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# Biomodulators effect in the transformation of fibroblasts to myofibroblasts in an experimental model *in vivo*

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**INTRODUCTION**: A key event in both repair and pathological fibrosis is the activation of fibroblasts and their conversion to myofibroblasts characterized by the expression of α-smooth muscle actin, so the design and search for new treatments that inhibit such event is imperative. There have been reported biological effects of the biomodulators on the modification in fibroblasts cell proliferation *in vitro* and on plant and animal stem cells differentiation, which proposes a therapeutic window to inhibit activation and proliferation of myofibroblasts.
**OBJECTIVE**: To Determine the inhibitory or stimulatory effect of biomodulators on the transformation of fibroblasts into myofibroblasts and on the α-actin and TGF-β expression in a granuloma model *in vivo*.
**MATERIALS AND METHODS**: A fibrosis rat model (granuloma) was developed to analyze the transformation of fibroblasts into myofibroblasts detecting the expression of α-smooth muscle actin, by immunohistochemistry. The animals were classified into six main groups, divided into three subgroups (n = 12) treated with different doses of the biomodulators specified in Table 1 and their respective controls. 8 visual fields of granuloma were quantified by using the software Image J. The comparison of means was analyzed by Tukey's test using SPSS. Subsequently extraction and protein quantification were performed in such granulomas and the detection of α-smooth muscle actin and TGF-β by Western Blot. Densitometric analysis was performed using the Image J program and comparison of means was analyzed by Tukey's test using SPSS.

Table 1. Biomodulators Concentrations

| BIOMODULATOR | GROUP 1 | GROUP 2 | GROUP 3 |
|---|---|---|---|
| 2,4-Dichlorophenoxyacetic acid (2,4-D) | 0.0452 mM | 0.00452 mM | 0.000452 mM |
| Indole-3-acetic acid (IAA) | 0.1 mM | 0.05 mM | 0.025 mM |
| 2-*Isopentenyladenine (2-IPA)* | 0.1 mM | 0.01 mM | 0.001 mM |
| 1-Naphthaleneacetic acid (NAA) | 1.0 mM | 0.1 mM | 0.01 mM |
| 6-Benzylaminopurine (6-BAP) | 0.1 mM | 0.01 mM | 0.001 mM |
| Abscisic acid (ABA) | 0.1 mM | 0.01 mM | 0.001 mM |

**RESULTS**: Immunochemistry method. An inhibitory effect of fibroblast transformation into myofibroblast with IAA at 0.1mM and 0.05mm was observed, however a stimulator effect with 2,4-D at 0.0452mM, 2-IPA at 0.1mM, IAA at 0.025Mm, NAA at 1mM and 0.1mM.

Western Blot. A statistically significant inhibitory effect on the expression of α-actin subsequent to treatment with 2,4-D at 0.0452 mM, 0.00452 mM and 0.000452 mM was observed, 6-BAP at 0.01 mM and 0.001 mM, NAA at 0.1 mM and IAA at 0.025 mM with respect to the control, while for 2-IPA and ABA concentrations employed showed no statistically significant effect. A statistically significant stimulatory effect on TGF-β expression following treatment with NAA at 0.01 mM relative to the control was observed, while for 2,4-D, IAA, 2-IPA, ABA and 6-BAP concentrations employed showed no statistically significant effect.
**CONCLUSIONS:** We recommend that the transformation of these cell types can be regulated by the biomodulators analyzed in this project.

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# Effect of fibronectin on acrosome reaction of guinea pig sperm

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Sperm capacitation occurs in the oviduct, where several molecules contain in the oviductal fluid interact with the sperm. Apparently, these molecules modulate sperm capacitation by mechanisms not yet defined. It suggests that some molecules present in the oviductal fluid and from cumulus oophorus could be involved in modulating sperm physiology and acquisition of the fertilizing capacity of sperm. Among these molecules is included the protein fibronectin. Fibronectin is a component of the extracellular glycoprotein and present in follicular fluid and oviductal matrix. Fibronectin is also found in the oviductal epithelium, on the luminal surface of the ciliated cells. Integrins mediate the linkage of the cell to Fibronectin; specifically, the α5β1 integrin through the RGD (Arg-Gly-Asp) domain. Interestingly, the α5β1 integrin is present in mammalian sperm. The integrin linkage to extracellular matrix proteins triggers different transduction pathways signals such as those related to increase the intracellular concentration of Ca2+, activation of kinases such as PKA, IPK3, AKT, PKC and Src, which have been involved in the regulation of sperm function. Interestingly, Fibronectin has been found linked to the surface of mammalian sperm. However, its role in capacitation and acrosome reaction is unknown. Additionally, it also knows that during capacitation, sperms are aggregated through their heads (rosettes).Therefore, the purpose of this study was to determine the participation of Fibronectin on sperm surface in acrosome reaction of guinea pig sperm. The tripeptide RGD considered as an inhibitor of the interaction of integrins with extracellular matrix proteins. Therefore, sperms were incubated under the condition of capacitation in the presence of different concentration of the RGD tripeptide (0-100 μM). Evaluation of the acrosome reaction shows that RGD had no effects on acrosome reaction. While, acrosome reaction increased in a dose-dependent manner when sperms were incubated under the condition of capacitation at different times (0, 30 60 90 and 120 minutes) and in the presence of different concentrations of Fn (0-100 ug /ml). However, the quantification of acrosome reaction of sperms incubated under capacitation conditions in the presence of the tripeptide RGD (25 μM) and FN (50 μg/ml), shows that the RGD tripeptide inhibited Fibronectin effects on the acrosome reaction. The results shown in this study indicate that the Fibronectin linked to the sperm surface could have no effect on capacitation and acrosome reaction.

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# Effect of Fatty acids in adipogenesis

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Obesity is a public health problem that has been associated with various diseases such as insulin resistance, diabetes type 2, dyslipidemia, non-alcoholic fatty liver disease, hypertension and cardiovascular diseases (Meigs, 2003; Wynne, 2005) Altogether, this diseases (including obesity) are referred to as the Metabolic Syndrome (MS) (Avogaro *et al*., 1967; Kim and Reaven, 2004; Eckel *et al*., 2005; Reaven, 2005; Cornier *et al*., 2008; Giorginio, 2009). The origin of the MS is multifactorial, mostly determined by physical inactivity and an unbalanced diet. It has been suggested that a Western fat-rich diet, results in the accumulation of lipids and adipose tissue even on organs (Unger RH, 200). The adipose tissue is an organ comprised of different cell types: fat cells, vascular endothelial cells, fibroblasts and macrophages (Nedergaard *et al*., 2007), secreting considered by the release of various hormones. Adipose tissue has been classified as white fat stores energy and brown fat involved in heat generation (Lowell and Flier, 1997). The adipose tissue also secrets several hormones like leptin, adipokines, interleukines and TNFα. Adipocytes represent two-thirds of adipose tissue and are specialized in the storage of fat cells (Heine *et al*., 200). Fatty acids are important in the body, as precursor molecules of phospholipids and sphingolipids which constitute part of the lipid bilayer of cell membranes. They are also energy reservoirs stored in the form of triacylglycerol in adipocytes. On the other hand, it has been observed that they can act as signaling molecules (McPhail *et al*., 1984; Ordway RN *et al*., 1991) whose role is of second messengers in the transcription of some genes. In this regard, the role of fatty acids has been little studied, so it is relevant to identify if fatty acids as palmitate (saturated) and oleate (unsaturated), which constitute the species with greatest presence in plasma as well as the palmitoleate (the main predecessor of polyunsaturated fatty acids) may have an impact in the adipogenesis and the metabolic status of the fat cells.

Using the model of adipose differentiation in cells 3T3-F442A (Marsch-Moreno and Kuri-Harcuch, 1983) established in our laboratory; we analyze the effect of both fatty acids: saturated as unsaturated, in the adipogenesis. Our results show that the presence of both types of fatty acids during the adipogenesis increases the number of cells and promotes the accumulation of lipids. In addition, we analyzed the expression of several genes involved in the secretory function of the adipocyte, insulin resistance, pro-inflammatory state, and adipogenesis. So far, the unsaturated fatty acids appear to improve insulin signaling. Conversely, in the presence of saturated fatty acids we have not found relevant changes. Given the importance that this may have on insulin resistance, further experiments are being done, whereby adipocytes differentiated in the presence of different fatty acids will be subjected to a treatment with TNFα and changes in gene expression will be analyzed.

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# Apoptosis Analysis of a Lung Cancer Cell Line Resistant to Radiotherapy

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*Lung cancer* is the *most common cause* of *death* from *cancer worldwide.* Despite the improvements in diagnosis and therapies, the prognosis and outcome of patients with lung cancer are still unsatisfactory. Radiotherapy is a treatment option for lung cancer patients, which achieves its therapeutic effects by inducing apoptosis. Unfortunately, a subpopulation of cells within the primary tumor avoids the cytotoxic effects of radiotherapy, permitting selection clonal of cancer cells with a more malignant phenotype and thus limiting the effect of treatment. One of the radioresistance mechanisms of the tumor cells is avoid the apoptosis signals. Therefore the study of apoptosis pathways will clarify the molecular mechanisms of radioresistance in cancer cells. In an effort to understand the variation in apoptosis pathways in radioresistance cells we established the Calu-1 lung cancer cell line radioresistant (Calu-1RR) by continuously exposure to ionizing radiation. We confirm the resistance to radiotherapy of the Calu-1RR cells by clonogenic assays. For to induced apoptosis, Calu-1RR and Calu-1 cells were treated with 150 J/m²s of UV-C light and apoptosis levels were evaluated by TUNEL and Annexin V assays. Results of TUNEL assays showed that Calu-1RR cells display low damage of DNA 20 % compared to control Calu-1 cells 32 %. Moreover, the results of Annexin V showed lower percentage of Calu-1RR cell (2%) with phosphatydilserine translocate compared to parental Calu-1 cells (34%). These results indicated that Calu-1RR cells are refractory to apoptosis signals. In order to clarify the pathways of the apoptosis, the expression and activation of caspases and Bcl-2 proteins family will be evaluated by western blot. We conclude that Calu-1RR cells could be a good biological model to study the resistance to radiotherapy in lung cancer. The perspectives of this work is to analyze the regulatory proteins of apoptosis in Calu-1RR cells exposes to ionizing radiation and to explore the survival pathways. It is of great importance to understand the molecular mechanisms of the radioresistance for to identified biomarkers and therapeutics targets.

# MPK6 loss of function effects on the pattern of expression of the transcription factor ABI4 during embryo and root development and in response to ABA and glutamate in *Arabidopsis thaliana.**

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The transcription factor ABI4 and the kinase MAP6 of *A. thaliana*, have been associated with signal transduction pathways controling embryo (1) and root (2) development programs, as well as, responses to abscisic acid (ABA; 3) and glutamate (L-Glu; 4). Besides, through a global phosphorylation study it was reported that ABI4 is a target of MPK6 (5), a result that has been corroborated by phosphorylation gel assays in our laboratory. Based on those function matches, we decided to explore the effect of the loss of function of MAP kinase 6 on the pattern expression of ABI4 during different stages of development and in response to treatments with ABA and L-Glu. So, to monitor the expression pattern of the ABI4' promoter, a reporter construction (ABI4::GUS; 6), was introgressed by controlled crosses in the MPK6 mutan background (2). The results show that the activity of the protein MPK6 is not required for the ABI4 expression during embryonic and early seedling development. As MPK6 is not expressed in embryos or roots, this suggests that their participation in these development programs, if any, is cell autonomous. Regarding both genes responses to treatments with ABA and L-Glu, both regulators induce expression ABI4 slightly, even if these responses are independent of MPK6 activity. Meanwhile, the pattern expression of MPK6 is not altered by the application of ABA or L-Glu. Taken together, our results suggests that if ABI4 needs to be phosphorylated to exercise its function in the processes described, most likely that phosphorylation is performed by any other member of the family of MAPKs of *A. thaliana* whose functional redundancy is very well demonstrated (7).

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# Study of Ski Transcriptional Cofactor Stability

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Ski is an oncoprotein broadly characterized by its inhibitory effect on TGF-β signaling, since it acts as a transcriptional co-repressor of target genes that are activated by this pathway. TGF-β can regulate several cellular processes like differentiation, migration, proliferation, and others, and its responses vary in cell type and context-dependent way, so it becomes relevant the study of Ski protein regulation and its influence on TGF-β signaling.

The activation of TGF-β signaling promotes Ski protein degradation via proteasome; Ski protein stability is also regulated during cellular mitosis, in which Ski increases its levels by association with certain kinases that control the cell cycle. Recently, we reported the Ski interaction with actin, a protein of the cytoskeleton that can associate with Ski in its globular form and it enhances Ski protein stability, this fact is related with a cytoplasmic localization of Ski in multivesicular bodies, despite Ski has been described mainly as a nuclear protein. This suggests that cytosolic Ski could have different functions and mechanisms of regulation yet unknown.

These mechanisms of regulation suggest that Ski undergoes post-translational modifications (PTMs). To cite some examples, Ski degradation by proteasome involves Ski ubiquitination; in the association with kinases of cell cycle, Ski could be phosphorylated, and others PTMs may also regulate its cellular localization. However, the characterization of these PTMs still remains to be studied.

In our research group, we have identified potential sites of PTMs along Ski amino acid sequence by *in silico* analysis; these include phosphorylation, ubiquitination, sumoylation palmitoylation and glycosilation. Most of these predictions are localized in the carboxi terminus, in which the sequence is less conserved between Ski and others members of the family like SnoN; by contrast, the amino terminus contains regions highly conserved and whose possible modified sites are less abundant.

Experimentally, we use C9 hepatic cells to study different mechanisms that control Ski protein stability and subcellular localization in order to identify any possible PTM and its impact on Ski regulation. At the moment, we have confirmed by Western blot that Ski is degraded via proteasome after TGF-β signaling, but still we have not identified its ubiquitination sites. In addition, the WB showed that Ski protein runs mainly as three bands that have been associated to different isoforms that differ in size by approximately 10 KDa; to explain this fact, an analysis of possible splicing has been done and discarded, so this strengthens the idea that Ski undergoes PTMs.

These results give the basis to do a more detailed characterization of cellular process involved in Ski stabilization and/or in its subcellular localization.

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# Isomers of conjugated linoleic acid induce insulin resistance through pkcε activation in hepatic cells

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Metabolic syndrome describes a group of abnormalities including hypertension, central obesity, insulin resistance, and hypertriglyceridemia. Alteration in the composition of fatty acids in the diet may participate in these states; diets that are rich in unsaturated fatty acids counteract this effect. Conjugated linoleic acid (CLA) constitutes a group of isomers of linoleic acid, a polyunsaturated fatty acid. The role of CLA isomers in their anti-inflammatory effects makes CLA a primary candidate for dietary treatment in states of insulin resistance[1]. However, it has been proposed that anti-inflammatory and anti-diabetic effects of CLA are isomer specific. While the cis-9, trans-11 isomer is responsible for reducing levels of pro-inflammatory cytokines and insulin resistance in adipose tissue cells, the trans-10, cis-12 isomer promotes insulin resistance, reduction of body fat accumulation, hepatic steatosis, and increase in inflammatory interleukins[2], effects that could be contradictory. Hepatic insulin resistance could be attributed to the activation of PKCε, which was the predominant PKC isoform activated in the liver following fat feeding[3]. This effect was associated with decreased insulin-stimulated insulin receptor substrate (IRS) tyrosine phosphorylation by the insulin receptor, leading to the inability of insulin to activate hepatic glycogen synthesis and suppress hepatic glucose production. A few reports have shown that insulin receptor expression is inversely correlated with PKCε activation in obese diabetic rats[4] and human HepG2 cell line. Moreover, lipid-induced hepatic insulin resistance could be prevented by knocking down PKCε[5]. Dietary CLA increases hepatic DAG, membrane-associated PKCε, and promotes the pathophysiology responsible for the development of hepatic insulin resistance. Because the molecular mechanisms of PKCε activation in hepatic insulin resistance induced by CLA are still unknown, it is relevant to investigate the relationship between the effects of this fatty acids on insulin signaling pathway and the role of PKCε in hepatic cells. Our results clearly show that the two isomers of CLA decrease insulin-stimulated phosphorylation of key proteins involve in insulin signaling such as Akt at $Ser^{473}$ and $Thr^{308}$, the insulin receptor at $Tyr^{1158}$, IRS-1 at $Tyr^{632}$ and GSK-3 at $Ser^{9/21}$. However, the protein expression was unaffected. Interestingly, both isomers of CLA promote phosphorylation and activation of PKCε. When a dominant negative of PKCε was transfected in C9 cells, the negative effects of CLA on Akt phosphorylation were improved. Additionally, we also found that both isomers of CLA increase phosphorylation of IRS at $Ser^{612}$, mechanism that probably underlies the inhibition of IRS signaling by PKCε[6]. These findings suggest that the two main isomers of CLA could have a significant role in the development of insulin resistance in hepatic cells through IRS serine phosphorylation and the PKCε activation. This work was supported by CONACYT grant 167673 to JAOR and CONACYT scholarship 245147 to ARG.

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# Interaction And Regulation Of Retinoblastoma Protein By Mdm2/Mdmx Proteins In Different Conditions.

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The retinoblastoma protein (Rb) plays a critical role in cell cycle regulation and tumor suppression. In its hypo-phosphorylated state, Rb is active and prevents cell cycle progression by binding to the E2F transcription factor. When Rb is phosphorylated by cyclin-dependent kinases (CDKs) is inactive, allowing cells to go through G1 to S phase. Inactivation of Rb promotes continuous cell division leading to different cancers, including retinoblastoma. Have been reported that, in retina cancer the oncoproteins MDMX and MDM2 are overexpressed. MDM2 promotes the ubiquitination and subsequent degradation of Rb, while MDMX stabilizes MDM2 under certain cellular conditions and promotes its ubiquitin ligase activity. Under genotoxic stress conditions, MDMX and MDM2 are ATM-dependent phosphorylated while Rb is p38-dependent phosphorylated under the same conditions. These phosphorylation events provoke conformational change that could stimulate protein-protein interactions. In this study, our interest is to study post-translational modifications, particularly phosphorylation in MDM2, MDMX and Rb and their role to promote the formation of a ternary complex between RB-MDM2-MDMX and the role that p53. First we produce and purify Rb in recombinant systems, testing two different *E.coli* strains (BL21 and Rosetta) and different culture conditions (temperature and IPTG concentration). *In vitro* Rb ubiquitination assay with MDM2 will be carried out. The influence of MDMX in this process will be also studied.

# Toll-like receptor 4 activation by lipopolysaccharide promotes progression in breast cancer

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Toll-like receptors (TLRs) are members of the interleukin-1 receptor (IL-1R) superfamily that share significant homology in their cytoplasmic regions, the Toll/IL-1R (TIR) domain. TLRs 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. TLR4 has already been linked to tumors such as ovarian, prostate and head and neck cancers. However, little research has investigated the role of TLR4 in breast cancer progression.

This study investigated the expression and biological role of TLR4 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 of IL-6, TNF-$\alpha$, epithelial-mesenchymal transition (EMT)-related genes (*SNAIL-1*, *SNAIL-2* and *ZEB-1*), Nav 1.5 sodium channel $\alpha$ subunit. LPS enhanced migration of MDA-MB-231 cells by transwell assay and wound healing assay. LPS triggered increased expression of TLR4 downstream signaling pathway protein myeloid differentiation factor 88(MyD88) and NF$\kappa$-B.

These findings indicated that TLR4 may participate in the progression and metastasis of human breast cancer and provide a new therapeutic target.

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# Effect of obesity on vascular reactivity and $Ca^{2+}$ homeostasis in *in situ* endothelial cells from female rat aorta

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Introduction: Obesity is an important public health problem in worldwide, is considered one of the main cardiovascular risk factor, increasing morbidity and mortality from hypertension, heart failure, stroke, and coronary artery disease. Abnormal vascular reactivity and endothelial dysfunction are known to be early events that underlies the development of subsequent cardiovascular diseases found in obesity. Endothelial cells are involved in a diverse range of functions including, the production of antithrombotic, pro-coagulant, inflammatory mediators and growth factors, as well as control of vasomotor tone. Most of these endothelial functions are tightly regulated by intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$). **Justification:** Despite the available evidences of a link between $[Ca^{2+}]_i$ and endothelial cell function, as well as documented endothelial dysfunction in obesity, there are not studies correlating the vascular reactivity with $Ca^{2+}$ signaling in *in situ* endothelial cells (IEC) of obese animal models. **Aim:** To determine the effect of obesity on the vascular reactivity and $[Ca^{2+}]_i$ in IEC from female Zucker Diabetic Fatty (ZDF) rat aorta. **Methodology:** Thoracic aorta was extracted, cut in 5mm rings and connective tissue was removed. Vascular reactivity measurements were performed using isometric tension bath chamber, for $Ca^{2+}$ signal recording, aortic rings were opened, loaded with Fura-2, and measured by microfluorimetric techniques using a direct microscope. **Results:** Female obese ZDF rats presented a significant increase in: a) body weight (170%) b) abdominal circumference (134.84%) and c) periabdominal adipose tissue (765.44%) compared with control rats, clearly confirming its obese phenotype. Non-significant differences were found in the glucose tolerant curve test between rat groups. Vascular reactivity in response to norepinephrine was increased by 201.5% in obese aortic ring with intact endothelium, and by 133% in obese aortic rings without endothelium. Vascular reactivity increase was not due to the nitric oxide bioavailability alterations because the relaxing effect of acetylcholine was similar in aortic rings obtained from obese and control rats. The application of adenosine triphosphate (ATP) 20µM to IEC evoked a $Ca^{2+}$ signal consisting in a rapid increase in $[Ca^{2+}]_i$ (peak) followed by a slow decay of the $[Ca^{2+}]_i$ (decay) to the baseline, however the peak amplitude was increased significantly in obese IEC. In addition, $Ca^{2+}$ entry though store operated $Ca^{2+}$ channels was increased in endothelium from obese rats. **Conclusion:** Obesity caused alterations in vascular contractility and endothelial $Ca^{2+}$ homeostasis in rat aorta.

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# Study of the regulation of the PI3-kinase/Akt pathway by the *O*-GlcNAcylation in cell lines derived from cervical cáncer

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Introduction: Cervical cancer is the second most prevalent cancer in Mexico. Signaling PI3-kinase/Akt pathway is important in regulating metabolism, proliferation, survival, migration and apoptosis. Previous studies suggest that Akt may suffer both phosphorylation and O-GlcNAcylation and balance between these modifications can regulate different cellular functions. The O-GlcNAcylation is a reversible dynamic posttranslational modification, which involves the addition of GlcNAc residues to Ser/Thr cytosolic, nuclear and mitochondrial proteins, catalysed by OGT and eliminated by the OGA. The aim of this work is to study the regulation of PI3-kinase / Akt pathway by O-GlcNAcylation.

Methodology: cervical cancer cell lines were used to assess the expression of Akt-P, OGT, and OGA subsequent incubation with O-GlcNAcylation inducers by immunocytochemistry assays and western blot.

Results: The expression of the O-GlcNAcylation with subcellular localization in the nucleus and cytoplasm was observed, as well as expression of OGA and OGT enzymes in all cell lines under basal conditions citoquímica cytometry and western blot. By using inducers O-GlcNAcylation increased this modification associated with increased and relocation of Akt-P to the cell membrane, likewise one colocalization of the O-GlcNAcylation with Akt-P observed by cytochemistry was found by western blot increased phosphorylation of Akt using inducers of O-GlcNAc was observed. Conclusions: There is expression of the O-GlcNAcylation, OGT and OGA in cervical cancer cell lines; in the same way results suggest that O-GlcNAcilación upregulates PI3-kinase / Akt pathway by Akt phosphorylation.

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# Function of the Peroxisomal proteins Pex1, Pex4, Pex6 and Pex8 in the sexual development of the filamentous fungus *Podospora anserina*

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Peroxisomes are cellular organelles which are found in eukaryotic cells. They serve in different cellular process and the most important are lipid metabolism and protection against reactive oxygen species. Peroxisome biogenesis requires a group of proteins derived from ER, which are called "peroxins". Particularly there are a group of peroxins involved in the formation of a pore in the peroxisomal membrane through which peroxisomes translocate proteins essential for different metabolic and cellular processes. This molecular complex designed "importomer" could be divided into 3 parts: the pore complex, ring complex and export /recycling complex. The import of proteins into the lumen of the peroxisome follow two main pathways which are recognized by two signals: the C-terminal (PTS1) and the N-terminal (PTS2) whose recognition depends on Pex5 and PEX7 / Pex20 receptor proteins respectively. In the filamentous fungus *P. anserina* mutants in the ring complex proteins affect the sexual development by meiosis block, however, surprisingly mutants in the receptor proteins Pex5, PEX7 and the pore forming PEX14 protein don´t have any defect in the sexual development of the fungus. These facts have led to proposing a third import mechanism mediated by the Pex20 protein, because double or triple mutants with Pex5 and Pex7 generate a phenotype similar to the ring complex proteins affecting sexual development. In this work we analyzed the phenotype of mutants in the peroxins Pex1, Pex4, Pex6 and Pex8 which are responsible for an impairment in the sexual development by blocking meiosis, likewise, all the mutants are affected in the import TPS1 and TPS2 pathway. These results suggest that the integrity of importomer/exportomer machinery is essential for the sexual development in *Podospora anserina*.

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# The actions of exogenous leucine on legume TARGET OF RAPAMYCIN signalling

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## ABSTRACT

Amino acids are essential for the regulation of cell growth and proliferation in two ways; by providing the substrate required for polypeptide biosynthesis, and by modulating signalling pathways responsible for protein synthesis. Amino acids are essential for mammalian target of rapamycin (mTOR) activation. Leucine is known to stimulate skeletal muscle growth via TOR signalling in animals. However, in plants it remains unclear how specific amino acids are sensed by TOR. The mechanism of leucine interactions with TOR in plants are needed urgently to unravel the novel functions of TOR. Thus, aim of the present study was (1) to investigate the effects of leucine on TOR signalling in model grain legume, *Phaseolus vulgaris* and (2) to understand the influence of leucine on the rhizobial root nodule development. To achieve this initially we grew wild type *P. vulgaris* in presence of exogenous leucine and later established the TOR expression levels by RT-qPCR. This study also presents the quantitative data on the rhizobial infection and nodule development in leucine treated roots. The expression profiles of different root growth, symbiotic related and TOR signaling genes provide the genetic elements that responded to leucine. Together, these results show the effects of exogenous leucine on TOR signalling and nodule symbiosis in *P. vulgaris*. This work is supported by CONACYT-240614 and partially by PAPIIT (DGAPA-UNAM) grant no. IN219916 to M.L.

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# Participation of Actin Depolymerization Factor D (ADFD) in the early stages of the *Phaseolus vulgaris – Rhizobium* symbiosis

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Common bean (*Phaseolus vulgaris*) is the most consumed legume by humans and is an essential source of proteins for the population of scarce economic resources, globally. Legumes are capable of establishing a symbiotic relationship with soil bacteria (*Rhizobium*). These bacteria interact with the roots of legumes inducing the formation of nodules, where biological nitrogen fixation occurs. This interaction starts with a molecular dialogue between both symbionts in which the bacteria synthesize and secrete lipochito-oligosaccharides, named “Nod factors” (NFs), in response to flavonoids produced by the roots of the legumes. In the first steps of infection, the bacteria enter the tip of the root hair inducing, among other responses, an increase in ions concentration as wells as deformations and microfilament reorganization. Then, infection threads (ITs) are formed and through these the dividing rhizobia penetrates to the root cortex where nodule primordia is forming and the bacteria are eventually released to the nodule.

Moreover, microtubules and microfilaments, such as actin filaments, are essential in the nodulation process as it has been reported over the years by our group. Basically, we have shown that there is a rearrangement of the actin cytoskeleton, minutes after NFs treatment[1,2,3]. Hence we are interested in studying actin remodeling proteins to determine their function during this symbiosis and given that Actin Depolimerization Factor (ADF) have been directly related to the increments in actin dissociation[4], we selected this protein for further characterization.

Hitherto, we identified 9 ADF genes in *Phaseolus vulgaris*, in which we selected ADFD based on its high transcript accumulation levels in root hairs. Currently we are working on the characterization, using a reverse genetic approach, of ADFD functions during the infection and nodulation process in bean. Therefore, in this work we are presenting the analysis of the loss of function (gene silencing) and overexpression phenotypes in terms of IT progression, nodule number/size and nitrogen fixation capacity. Additionally, we will present some evidence of ADFD-GFP localization pattern as well as the characterization of the activity of ADFD promoter region.

[1]Cárdenas 1998, 1999, 2008; [4]Co Gibbon 1997

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# Participation of STAT5 in the regulation of expression of GLUT-1 and hexokinase II

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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. One of the most important characteristics of many types of cancer is that present altered metabolism, enhanced glucose uptake and glycolysis, and decrease oxidative metabolism. Because this is essential to know the signaling pathways used by cervical carcinoma cells to maintain their malignant phenotype. In some normal cells (like T cells), the switch metabolic is maintained by the continuous signaling of IL-2.

Signal Transducer and Activator of Transcription (STAT) proteins are transcription factors essential for cellular response to cytokines and growth factors. Upon binding of a ligand to the receptor, become phosphorylated in tyrosine, dimerize, translocate to the nucleus, and regulate the expression of genes that modulates cellular functions. Increasing evidence suggest that STAT signaling may be involved in regulating cellular metabolism. In particular, some carcinoma cell lines

expressed the molecules JAK3 and STAT5 constitutively active, because we analyze whether the STAT5 molecule is involved in regulating genes engaged in energy metabolism such as glucose transporter 1 (GLUT-1) or hexokinase II.

The carcinoma cell lines HeLa and SiHa were transfected with siRNA directed against STAT5, and the cells were stimulated with IL-2 (10UI/mL), and RNAm was extracted. The RT-PCR was performed with 3μg of RNA, and posteriorly the PCR was made using primers to GLUT-1 and hexokinase II.

The presence of siRNA decreases the presence of STAT5, GLUT-1 and hexokinase II; the stimulation with IL-2 fails to increase the same molecules.

This work was supported by a grant from DGAPA, UNAM (PAPIIT IN222915).

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# Histamine Impairs Midbrain Dopamine Neuron Differentiation in Association to Modifications of Epigenetic DNA Marks.

Fernanda Vargas-Romero[1†], Ernesto Soto-Reyes.[2], Rodrigo González-Barrios[2], Lissania Guerra-Calderas[2], Iván Velasco[1*].

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During rat midbrain (MB) formation, dopamine neuron differentiation occurs between embryonic days (E) 9 to 15. During this process, the regulation of extrinsic and intrinsic signals that modulate the expression of different transcription factors at temporal and spatial levels, are fundamental. Modifications of epigenetic marks in histones and DNA are essential for the expression of genes required for dopaminergic differentiation. DNA demethylation process has an important role during neurogenesis, since increases of 5-hydroxymethylcitosine (5hmC) has been associated to transcriptional activation of neuronal genes. Recently, we demonstrated that injection of histamine (HA) in the cerebral ventricle at E12 decreases the number of mesencephalic dopamine neurons. However, the mechanism of HA´s action remains undefined. Here we show that ultrasound-guided injections of HA at E12 decrease the percentage of 5-methylcytosine (5mC) on the exons of *Pitx3* and *Th*, suggesting a role of HA on the transcriptional regulation of these genes. Using RT-qPCR, we found that HA decreases the expression of early genes involved in midbrain dopaminergic specification, such as *Lmx1a*, *Msx1* and *Foxa2* and importantly, also down-regulates the terminal differentiation genes *Pitx3* and *Th*. Thus, decreases of 5mC on *Pitx3* and *Th* and the lower levels of its transcripts, suggest an important role of HA during the regulation of transcription-splicing process. We also found that HA has a long-term effect on the dopaminergic axonal growth, since the administration of HA at E12 decreased the number of neuronal fibers positive to Tyrosine Hydroxylase in the nigrostriatal pathway, 6 days after the injection. These findings propose a molecular mechanism of action of HA during the development of dopaminergic neurons.

Supported by PAPIIT IN208713 / IN213716 and CONACyT CB09/131281.

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# Effect of Diabetes Mellitus Type 2 on the Regulation of Intracellular $Ca^{2+}$ in Excised Endothelial Cells from Rat Aorta

Verónica Ajelet Vargaz Guadarrama, José Alonso Romero, Josué Ernesto Sánchez Gómez, Francesco Moccia, Franco Tanzi, Roberto Berra Romani.

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**Introduction:** Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by increased blood glucose levels, affecting 180 million people worldwide, and this number is projected to reach 366 million by 2030. Cardiovascular disease is the leading cause of death among individuals with T2DM, accounting for up to 75% of all deaths in some studies. Endothelial dysfunction is known to be an early event that underlies the development of subsequent cardiovascular diseases found in T2DM. Endothelial functions such as adhesion, vessel growth and production of vasoactive substances are tightly regulated by intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$).
**Justification:** Despite the available evidences of a link between $[Ca^{2+}]_i$ and endothelial cell function, as well as documented endothelial dysfunction in diabetes, there are no studies analyzing the $Ca^{2+}$ signaling in excised endothelial cells (EEC) of animal models with T2DM.
**Aim:** To determine the effect of T2DM on the $[Ca^{2+}]_i$ in EEC from Zucker Diabetic Fatty rat aorta.
**Methodology:** Thoracic aorta was extracted, cut in 5mm rings and incubated with Fura 2-AM (16µM). Aortic rings were opened and $Ca^{2+}$ signal was measured by microfluorimetric techniques using a direct microscope. All experiments were performed in $Ca^{2+}$ free medium to study the $Ca^{2+}$ signal arising only from intracellular stores.
**Results:** The application of adenosine triphosphate (ATP) 300µM to EEC, evoked a $Ca^{2+}$ signal consisting in a rapid increase in $[Ca^{2+}]_i$ (peak) followed by a slow decay of the $[Ca^{2+}]_i$ (decay) to the baseline. Similar response was observed in control and diabetic EEC. However, when sarcoplasmic-reticulum-$Ca^{2+}$-ATPase (SERCA) and plasma-membrane-$Ca^{2+}$-ATPase (PMCA) were selectively inhibited by cyclopiazonic acid (CPA, 10µM) and (CEDA, 20µM), respectively, the $Ca^{2+}$ transient peak amplitude and decay time, were increased significantly in diabetic EEC. Using KB-R7943 (20µM), as a specific inhibitor of the $Na^+/Ca^{2+}$ exchanger (NCX), did not modified the $Ca^{2+}$ response evoked by ATP. **Conclusions:** T2DM resulted in significant alterations in SERCA and PMCA activities in EEC from rat aorta; while no differences in NCX activity between diabetic and wild-type cells were observed.

# Effect of the adenosine derivative compound IFC-305 on activated Hepatic Stellate Cells.

Gabriela Velasco-Loyden, Mariana Domínguez-López, Jesús Rafael Rodríguez-Aguilera, Julio Isael Pérez-Carreón 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.

In normal liver, hepatic stellate cells (HSC) are quiescent cells with large vitamin A lipid droplets and an adipogenic phenotype. During the development of liver fibrosis these cells become activated characterized by increased proliferation and excessive extracellular matrix protein synthesis. The aspartate salt of adenosine (IFC-305) prevents and reverses pre-established $CCl_4$-induced cirrhosis in rats (Perez-Carreon et al. 2010). We have previously shown that this compound suppresses the *in vitro* activation of rat quiescent HSC by inhibiting $\alpha$-SMA and collagen $\alpha$1(I) expression, and up-regulating PPAR$\gamma$, MMP13, and Smad7 expression (Velasco-Loyden et al. 2010). The aim of this work was to characterize the effect of IFC-305 on already activated HSC.

HSC were isolated from normal male Wistar rats and cultivated for 7 days to activate them, then they were incubated in the presence of IFC-305 for 48 hours or in a continuous treatment for the following 7 days. Effect of IFC-305 on HSC proliferation was evaluated by MTT assay and BrdU incorporation. Expression of proteins or mRNA was assessed by western blot or qRT-PCR.

IFC-305 reduced the proliferation of activated HSC in a dose dependent manner. In addition, IFC-305 blocked the expression of the pro-fibrogenic markers collagen $\alpha$1(I), TGF-$\beta$1, up-regulated the expression of the anti-fibrogenic gene PPAR$\gamma$ and acquired some adipogenic characteristics.

These results, together with the effect of IFC-305 previously described for quiescent HSC, suggest that the anti-fibrotic mechanisms of action of IFC-305 involves suppression of HSC activation as well as inhibition of some fibrogenic characteristics of activated HSC, with a possible reversion effect. These findings could explain in part the anti-fibrotic beneficial effect of IFC-305 on $CCl_4$-induced cirrhosis in rats.

*Thanks to PAPIIT of DGAPA- UNAM for economy support*

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# Expression of a specific peptide from SymHSP75, a light responsive protein from *Symbiodinium microadriaticum* , for polyclonal antibody raising.

Marco A. Villanueva Méndez, Raúl Eduardo Castillo Medina and Tania T. Islas Flores.

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, Q. Roo, México. Email: marco@cmarl.unam.mx

*Symbiodinium* microalgae belong to a genus of photosynthetic dinoflagellates that usually live as endosymbionts in marine invertebrates such as anemones, jellyfish and corals. However, they are also able to live outside their host as free-living planktonic organisms. Because of their photosynthetic nature they are sensitive to variations in lighting conditions from their environment. SymHSP75 was previously identified from *Symbiodinium microadriaticum* as a HSP90-like protein of ~ 75 kDa that is maximally phosphorylated on threonine after 12h of growth under continuous darkness. The original level of phosphorylation is observed to decrease when *Symbiodinium* cells are exposed to 30 min of light and this variation appears to depend on the intensity of the light stimulus. Interestingly, a low level of phosphorylation of SymHSP75 was observed when the endosymbionts were freshly isolated from their specific host previously adapted to 12h darkness. From an EST database of this microorganism, seven proteins of the HSP90 protein family were identified and one of them deemed as the most likely homolog of SymHSP75. The expression of a specific fragment from this sequence was achieved and used as antigen to generate specific polyclonal antibodies to SymHSP75. These antibodies immunodetected a ~ 75 kDa protein apparently corresponding to SymHSP75. Subsequent analysis with these antibodies will allow us to compare the expression of the protein and its phosphorylation level under darkness and upon the light stimulus. We will also focus on the determination of its subcellular localization and identity of some of its ligands. All these data will be crucial for the characterization of the role of this protein in *Symbiodinium*.

Supported by grants IN-210514 from PAPIIT DGAPA-UNAM and 175951 from CONACyT, to MAV; RECM was supported by the doctoral fellowship No. 255464 from CONACyT and belongs to the graduate program in Marine Science and Limnology of UNAM.

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# Suppression of the extracellular ATP (eATP) triggered host defense response by the root endophytic fungus *Piriformospora indica*

Shadab Nizam[1,2] and Alga Zuccaro[1,2#]

[1]Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch Strasse 10, 35043, Marburg, Germany

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## Abstract

The extracellular ATP (eATP) works as an extracellular signaling molecule in plants and animals. In plants, eATP triggers the production of reactive oxygen species (ROS), nitric oxide, callose deposition, transient phosphorylation of MPK3 and MPK6, and expression of genes involved in plant stress response and immunity. Furthermore, eATP is actively released from plant cells in response to abiotic stresses, fungal elicitors, and mechanical stimuli. Recently, transcript profiling has indicated that there is a considerable overlap of genes induced by ATP and by pathogens. Therefore, it appears that eATP plays a central role in mediating the plant immune response and thus can serve as a prime target for the pathogen assault. Indeed, it was shown that a *Phytophthora brassicae* secreted effector protein (IPI-O), containing the RXLR-dEER, targets LecRK-I.9 (also known as DORN1, a recently identified ATP receptor in *Arabidopsis thaliana*). LecRK-I.9 mutant plants showed enhanced susceptibility to the oomycete pathogen, whereas its over-expression resulted in increased resistance. However, nothing is clear about the role of eATP and its suppression during plant-fungus interactions. The filamentous root endophyte *Piriformospora indica* colonizes the roots of a wide variety of unrelated plants, including the dicot model plant *A. thaliana* and the monocot cereal crop *Hordeum vulgare* (barley). The fungus displays a biphasic colonization strategy with initial biotrophic phase followed by a cell death associated phase. Colonization by *P. indica* exhibits various beneficial effects on the host plant such as enhanced growth, tolerance to abiotic stresses, resistance against pathogens, and enhanced assimilation of nitrate and phosphate. Our results suggest that colonization of *P. indica* in *A. thaliana* and barley triggers the release of eATP. Furthermore, colonization of *P. indica* in the *A. thaliana* eATP receptor DORN1 (*dorn1*) mutant line clearly indicated that *dorn1* mutant supports significantly higher fungal colonization with respect to Col-0 control. Hence we expect that *P. indica* uses some intrinsic mechanism to hydrolyze the plant generated eATP, which eventually allows it to counteract ATP-mediated host immune responses. In order to identify candidate effectors during the interaction of *P. indica* with plants, the proteins present in the apoplastic fluid (APF) of barley roots colonized by *P. indica* at different symbiotic stages were analyzed by mass spectrometry. One of the most abundant identified peptide, at all the time points, was a predicted precursor for a 5'-Nucleotidase (5NT). The orthologue of this gene in human is an ecto-5'-nucleotidase (E5NT), a surface-located 5NT that functions as a part of an enzyme cascade, which sequentially and completely hydrolyses eATP to adenosine. Here we report *P. indica* E5NT as a candidate, possibly involved in controlling host eATP homeostasis.

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# Instructivo de navegación

Para ir a los resúmenes, solo hay que dar clic sobre el trabajo que queremos ver para regresar se da clic en el pie de pagina que dice:

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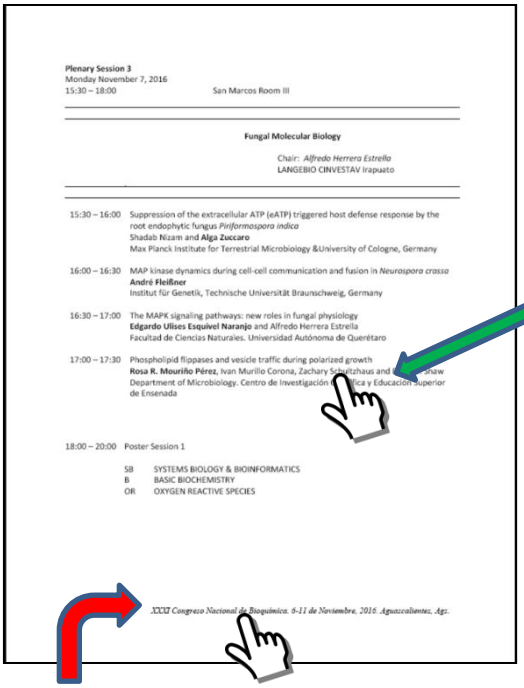

Plenary Session 3
Monday November 7, 2016
15:30 – 18:00 San Marcos Room III

**Fungal Molecular Biology**

*Chair: Alfredo Herrera Estrella*
LANGEBIO CINVESTAV Irapuato

15:30 – 16:00 Suppression of the extracellular ATP (eATP) triggered host defense response by the root endophytic fungus *Piriformospora indica*
Shadab Nizam and **Alga Zuccaro**
Max Planck Institute for Terrestrial Microbiology &University of Cologne, Germany

16:00 – 16:30 MAP kinase dynamics during cell-cell communication and fusion in *Neurospora crassa*
**André Fleißner**
Institut für Genetik, Technische Universität Braunschweig, Germany

16:30 – 17:00 The MAPK signaling pathways: new roles in fungal physiology
**Edgardo Ulises Esquivel Naranjo** and Alfredo Herrera Estrella
Facultad de Ciencias Naturales. Universidad Autónoma de Querétaro

17:00 – 17:30 Phospholipid flippases and vesicle traffic during polarized growth
**Rosa R. Mouriño Pérez**, Ivan Murillo Corona, Zachary Schaffhaus and [illegible] Shaw
Department of Microbiology. Centro de Investigación Científica y Educación Superior de Ensenada

18:00 – 20:00 Poster Session 1

SB SYSTEMS BIOLOGY & BIOINFORMATICS
B BASIC BIOCHEMISTRY
OR OXYGEN REACTIVE SPECIES

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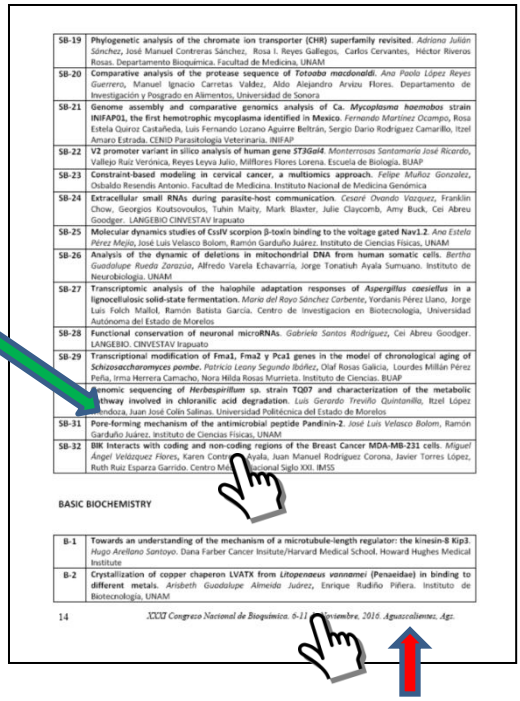

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|---|---|
| SB-19 | **Phylogenetic analysis of the chromate ion transporter (CHR) superfamily revisited**. *Adriana Julián Sánchez*, José Manuel Contreras Sánchez, Rosa I. Reyes Gallegos, Carlos Cervantes, Héctor Riveros Rosas. Departamento Bioquímica. Facultad de Medicina, UNAM |
| SB-20 | **Comparative analysis of the protease sequence of *Totoaba macdonaldi***. *Ana Paola López Reyes Guerrero*, Manuel Ignacio Carretas Valdez, Aldo Alejandro Arvizu Flores. Departamento de Investigación y Posgrado en Alimentos, Universidad de Sonora |
| SB-21 | **Genome assembly and comparative genomics analysis of Ca. *Mycoplasma haemobos* strain INIFAP01, the first hemotrophic mycoplasma identified in Mexico**. *Fernando Martínez Ocampo*, Rosa Estela Quiroz Castañeda, Luis Fernando Lozano Aguirre Beltrán, Sergio Dario Rodríguez Camarillo, Itzel Amaro Estrada. CENID Parasitología Veterinaria, INIFAP |
| SB-22 | **V2 promoter variant in silico analysis of human gene *ST3Gal4***. *Monterrosas Santomaria José Ricardo*, Vallejo Ruiz Verónica, Reyes Leyva Julio, Millflores Flores Lorena. Escuela de Biología. BUAP |
| SB-23 | **Constraint-based modeling in cervical cancer, a multiomics approach**. *Felipe Muñoz Gonzalez*, Osbaldo Resendis Antonio. Facultad de Medicina. Instituto Nacional de Medicina Genómica |
| SB-24 | **Extracellular small RNAs during parasite-host communication**. *Ceseré Ovando Vazquez*, Franklin Chow, Georgios Koutsovoulos, Tuhin Maity, Mark Blaxter, Julie Claycomb, Amy Buck, Cei Abreu Goodger. LANGEBIO CINVESTAV Irapuato |
| SB-25 | **Molecular dynamics studies of CssIV scorpion β-toxin binding to the voltage gated Nav1.2**. *Ana Estela Pérez Mejía*, José Luis Velasco Bolom, Ramón Garduño Juárez. Instituto de Ciencias Físicas, UNAM |
| SB-26 | **Analysis of the dynamic of deletions in mitochondrial DNA from human somatic cells**. *Bertha Guadalupe Rueda Zorazzia*, Alfredo Varela Echavarría, Jorge Tonatiuh Ayala Sumuano. Instituto de Neurobiología. UNAM |
| SB-27 | **Transcriptomic analysis of the halophile adaptation responses of *Aspergillus caesiellus* in a lignocellulosic solid-state fermentation**. *María del Rayo Sánchez Carbente*, Yordanis Pérez Llano, Jorge Luis Folch Mallol, Ramón Batista García. Centro de Investigacion en Biotecnologia, Universidad Autónoma del Estado de Morelos |
| SB-28 | **Functional conservation of neuronal microRNAs**. *Gabriela Santos Rodríguez*, Cei Abreu Goodger. LANGEBIO. CINVESTAV Irapuato |
| SB-29 | **Transcriptional modification of Fma1, Fma2 y Pca1 genes in the model of chronological aging of *Schizosaccharomyces pombe***. *Patricia Leany Segundo Ibáñez*, Olaf Rosas Galicia, Lourdes Millán Pérez Peña, Irma Herrera Camacho, Nora Hilda Rosas Murrieta. Instituto de Ciencias. BUAP |
| SB-30 | **Genomic sequencing of *Herbaspirillum* sp. strain TQ07 and characterization of the metabolic pathway involved in chlorinilic acid degradation**. *Luis Gerardo Treviño Quintanilla*, Itzel López Mendoza, Juan José Colín Salinas. Universidad Politécnica del Estado de Morelos |
| SB-31 | **Pore-forming mechanism of the antimicrobial peptide Pandinin-2**. *José Luis Velasco Bolom*, Ramón Garduño Juárez. Instituto de Ciencias Físicas, UNAM |
| SB-32 | **BIK Interacts with coding and non-coding regions of the Breast Cancer MDA-MB-231 cells**. *Miguel Angel Velázquez Flores*, Karen Cortés Ayala, Juan Manuel Rodríguez Corona, Javier Torres López, Ruth Ruiz Esparza Garrido. Centro Médico Nacional Siglo XXI. IMSS |

**BASIC BIOCHEMISTRY**

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|---|---|
| B-1 | **Towards an understanding of the mechanism of a microtubule-length regulator: the kinesin-8 Kip3**. *Hugo Arellano Santoyo*. Dana Farber Cancer Insitute/Harvard Medical School. Howard Hughes Medical Institute |
| B-2 | **Crystallization of copper chaperon LVATX from *Litopenaeus vannamei* (Penaeidae) in binding to different metals**. *Arisbeth Guadalupe Almeida Juárez*, Enrique Rudiño Piñera. Instituto de Biotecnología, UNAM |

14 *XXXI Congreso Nacional de Bioquímica. 6-11 de Noviembre, 2016. Aguascalientes, Ags.*

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**Suppression of the extracellular ATP (eATP) triggered host defense response by the root endophytic fungus *Piriformospora indica***

Shadab Nizam[1,2] and Alga Zuccaro[1,2#]
[1]Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch Strasse 10, 35043, Marburg, Germany
[2]University of Cologne, Botanical Institute, Cluster of Excellence on Plant Sciences (CEPLAS), Cologne Biocenter, Zülpicher Str. 47b, 50674 Cologne, Germany
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**Abstract**

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**G-proteins regulate mitotic spindle formation.**

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G protein-coupled receptors (GPCRs) are integral plasma membrane proteins that transduce fundamental messages related to perception, endocrine and paracrine communication. Upon ligand binding, GPCRs activate heterotrimeric G proteins by promoting GTP loading into Gα and dissociation of Gβγ. This initial event at the the plasma membrane is extended to intracellular compartments via vesicle trafficking. This spatiotemporal process provides a new dimension to Gα- and Gβγ-dependent signaling events, including a potential role in cell division. Gβγ, in particular, is known to interact with γ-tubulin, an important centrosome marker, indicating that Gβγ might control the assembly and progression of mitotic spindle. On the other hand, the endosomal sorting complexes required for transport (ESCRT) are involved in abscission of midbody during cytokinesis. ESCRTs are formed of 4 cytosolic protein complexes, known as ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III. We hypothesized that Gβγ links endosomal sorting complexes to the assembly of mitotic spindle. Thus, we tested whether ESCRT I (consisting of TSG101 (VPS23, vacuolar protein sorting 23), VPS37 and VPS28 proteins) is regulated by Gβγ. We found that Gβ1 interacts with VPS28, both in a yeast two hybrid system and in mammalian cells. This interaction has a prominent impact on mitotic spindle formation and does not require the activation of membrane receptors. Collectively, our results reveal a novel mechanism of communication between vesicle trafficking and the mitosis phase of the cell cycle, spatiotemporally regulated by Gβγ via its interaction with VPS28, a component of the sorting machinery.

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